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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office
Le Président de l'Office européen des brevets
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Fruit flavour related genes and use thereof

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12.03.1999 Fruit Flavour Related Genes And Use
Thereof

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The invention disclosed herein relates to strawberry (*fragaria ananassa*) and lemon (*citrus limon*) fruit derived 5 genes and enzymes specifically involved in the formation of aliphatic and/ or aromatic esters and other aroma and flavour compounds in fruits. More specifically it relates to a process for improving natural volatile fruit flavours by the control of one or more than one gene implicated in 10 that process.

Background of the invention

Like most natural products the chemical composition of strawberry aroma and flavour is quite complex [Zabetakis and Holden, (1997); J. Sci. Food. Agric 74: 421-434]. 15 Over 300 compounds have been identified which may contribute to flavour/aroma. Qualitatively, the major components of strawberry flavour and aroma may be grouped into several chemical classes including acids, aldehydes, ketones, alcohols, esters, and lactones. Other groups include 20 sulphur compounds, acetals, furans, phenols and even traces of epoxides, and hydrocarbons. Compounds produced by these groups, whilst often present at low levels, may have a significant impact on the overall flavour of strawberry.

In general, fruit flavour compounds develop 25 during ripening when the metabolism of the fruit changes to catabolism. Esters are proposed to be qualitatively and quantitatively the most important class of volatiles produced and are a key component of strawberry flavour. Seven volatile esters, ethyl hexanoate, methyl hexanoate, 30 ethyl butanoate, methyl butanoate, hexyl acetate, ethyl propionate and 2-hexenyl-acetate were reported to contribute largely to the aroma associated with the fruit. However, more than a 100 types of esters have been reported during the years to be identified in strawberry volatile analysis 35 [Zabetakis and Holden, (1997); J. Sci. Food. Agric 74: 421-434].

In lemon, the acetate esters derived from cyclic terpene alcohols are used frequently as flavour, fragrance, scent and aroma components. The lower fatty acid esters of acyclic terpene alcohols (geraniol, linalool, and citronellol) are the acetates (linalyl acetate, geranyl acetate) which are the main flavour components of lemon peel oils. These are used for obtaining citrus notes [Bauer, K and Garbe, D, (1985). Common fragrance and flavour materials. Preparation, properties and uses. VCH Publishers, Florida, USA]. Formates, propionates, butyrates occur less frequently. In addition to the cyclic and acyclic terpene esters, lemon is a rich source for the ester methyl anthranillate, which is a methyl ester of o-aminobenzoic acid. This compound is known to be a major constituent of *citrus limon* fruit and flowers and can also be found in the leaves.

Primary metabolites which include sugars, fatty acids, nucleotides and amino acids have been proposed to serve as precursors for the production of secondary metabolites such as volatile compounds. Sugars in the fruit in addition to acting as a carbon source, act as precursors for the flavour compound furanones [Zabetakis and Holden, (1996); Plant Cell Tissue and Organ Culture 45:25-29]. Amino acid metabolism leads to the formation of various aldehydes, acids, carbonyls, aliphatic and branched-chain alcohols, some of which serve as aroma and flavour components. The changes in amino acid content during maturation of fruits in relation to fruit flavour formation, have been studied in banana, tomato and strawberry. The amino acids valine, leucine and isoleucine have been proposed to serve as flavour precursors in banana fruit, and are metabolised to branched chain alcohols, 2-propanol, isoamyl alcohol, and 2-methylbutanol, respectively [Drawert and Berger, (1981); Bioflavour '81; Schreier, P., Ed.; de Gruyter: Berlin]. The conversion of alanine, leucine and valine to flavour compounds has also been demonstrated in tomato [Yu et al., (1968); Phytochemistry 6: 1457-1465]. In strawberry, alanine was proposed to be the main free amino acid

metabolised to flavour compounds. This was based mainly on the dramatic decrease in its content just before the formation of volatile aroma and flavour compounds commenced in the fruit [Perez et al., (1992); J.Agric. Food Chem. 40: 5 2232-2235]. In addition feeding alanine to strawberry cultures resulted in the formation of several esters such as methyl and ethyl hexanoate which are important constituents of strawberry volatiles [Drawert and Berger, (1981); Bioflavour '81; Schreier, P., Ed.; de Gruyter: Berlin]. 10 Like sugars and amino acids the fatty acids in strawberry play an important role in ester formation. In mango fruit there have been reports that fatty acids are actively metabolised during ripening. Changes in fatty acids and triglycerides have been associated with changes in aroma 15 and flavour during mango ripening [Gholop AS and Bandyopadhyay C, J Am. Oil Chem. Soc., 52: 514-516, 1975]. Therefore fatty acid related enzymes may have a role in the production of flavour volatiles. Thiolase is the last enzyme in the β -oxidation of fatty acids. It catalyses the thiolytic 20 cleavage involving a molecule of CoA. The products of this reaction are acetyl-CoA and acyl-CoA derivatives containing two carbon atoms less than the original acyl-CoA molecule that underwent oxidation. The acyl-CoA formed in the cleavage reaction may be utilised at the final stage of the 25 biosynthetic pathway for ester formation in fruit. Therefore the profile of fatty acid and amino acid precursors found in each fruit, along with the specificity of the enzymes in the biosynthetic pathway/process leading to ester formation could have a key role in determining the types of esters 30 formed.

A proposed biosynthetic pathway for volatile ester formation in fruits is illustrated in Figure 1. In fruits the transamination and oxidative decarboxylation of amino acids provides the precursors for volatile aroma compounds 35 such as aldehydes, acids, alcohols, esters and thiols. The transamination reaction is catalysed by aminotransferases. Aminotransferases or transaminases are enzymes promoting the

first step in the catabolism of L-amino acids by removing the α -amino groups. As a result of this transamination reaction, the α -amino group is transferred to the α -carbon atom of α -ketoglutarate, leaving behind the α -keto acid 5 analogue of the amino acid. Aminotransferases have been studied in various micro-organisms and some of them have been purified [Gelfand et al, (1997); J. Bacteriol. 130:429-440, Lee et al., (1985); J. Gen. Microbiol. 131: 459-467]. No strawberry fruit specific aminotransferase to date has 10 been cloned or characterised.

Ketoacids produced by transamination can be enzymatically degraded to the corresponding aldehydes or carboxylic acids. The enzyme which can catalyse this reaction is pyruvate decarboxylase. An EST encoding part of pyruvate 15 decarboxylase, the first to be characterised from a strawberry fruit has been mentioned in patent application WO 97/27295. The pyruvate decarboxylase enzyme provides precursors for the biosynthesis of volatile flavour compounds. Alcohol dehydrogenase enzymes have been implicated in the 20 interconversion of the aldehyde and alcohol forms of flavour volatiles. The mechanism of ester formation has been shown to be a coenzyme A dependent reaction. Esterification is a result of transacylation from acyl-coenzyme A to an alcohol. The enzyme is therefore termed as alcohol acyl transferase 25 (AAT) and plays a major role in the biosynthesis of volatile esters.

Ester formation by micro-organisms has been studied in most detail and has been cited in a number of publications [Harada et al., (1985); Plant Cell Physiol. 26(6): 1067-30 1074], Fujii et al., (1996); Yeast 12:593-598, Fujii et al., (1994); Applied and Enviro. Microbiology 8:2786-2792]. Alcohol acyl transferases (AAT's) have been identified from both fungi [Yoshihide et al., (1978); AGRIC. Biol. Chem. 42(2):269-274], and yeast [Fujii et al., (1994); Applied and 35 Enviro. Microbiology 8:2786-2792] and have been the subject of patent applications (EP 0 574 941 A2). The AAT from the fungi *Neurospora* sp. which was purified to homogeneity acts on various acyl Coenzyme A containing more than a four

carbon linear chain but not on acetyl coenzyme A as described for the yeast enzyme [Yamauchi et al., (1989); Agric. Biol. Chem. 53(6) 1551-1556]. In *Cladosporium cladosporioides* No. 9, AAT was partially purified and was described to 5 form acetate esters like the yeast enzyme [Yamakawa et al., (1978); Agric. Biol. Chem. 42(2):269-274]. So it is clear that the type of esters formed is dependant on the substrate specificity of the ester forming enzyme to alcohols, and the availability of acyl-CoAs. For example the yeast *Saccharomyces cerevisiae* (sake yeast) and brewer's yeast (bottom 10 fermenting yeast) AAT's show high affinity to acetyl-CoA and the alcohols ethanol and isoamyl alcohol, giving rise to the formation of acetate esters. These esters play an important role in determining flavour characteristics of beer.

15 In plants ester formation has been studied both in fruit and flowers. Melon, banana and strawberry AAT's have been investigated using crude fruit extracts. The formation of esters from aldehydes that were incubated with whole strawberry fruit has been reported [Yamashita, et al., (1989); Agr. Biol. Chem. 39(12) 2303-2307]. Analysis of the 20 substrate specificity of the enzymes from the various sources, revealed differences in their affinity to acyl-CoAs and alcohols [Ueda et al., (1992); Nippon Shokukin Kogyo Gakkaishi 39(2): 183-187, Perez et al., (1996); J. AgricFood 25 Chem. 44: 3286-3290, Harada et al., (1985); Plant Cell Physiol. 26(6): 1067-1074]. Maximum activity for the strawberry (AAT) was obtained using acetyl CoA and hexyl alcohol as substrates, and acetyl-CoA and butyl alcohol is the preferred substrate for the banana enzyme. A clear correlation 30 could be observed between substrate preference and volatile esters present in both fruits. The purification method and some properties of the strawberry AATase enzyme have been reported. However, to date no sequence of the peptide or of the gene has been disclosed. In *Clarkia breweri* the ester benzylacetate is an important constituent 35 of flower scent. The purification of the protein and the gene AAT encoding it has been reported. The flower AAT enzyme was not reported to have affinity to aliphatic

alcohols. The enzyme has high affinity to the aromatic alcohols such as benzyl alcohol and cinnamyl alcohol [Dudareva et al., (1998); *The Plant Journal* 14(3) 297-304]. Genes coding for flower aromatic acyl transferases, that acylate 5 plant pigments, causing changes in colour tone have been the subject of a recent patent application [EP 0810287]. Recently, an aromatic amino transferase enzyme has been purified from lactic acid bacteria and was shown to initiate the conversion of amino acids to cheese flavour compounds [Yvon 10 et al., (1997); *Applied and Environmental Microbiology* 414-419].

Although volatile esters are qualitatively and quantitatively one of the most important classes of volatile compounds in fruit flavour and aroma, there are very few 15 reports, concerning the biochemical aspects of ester formation in fruits. An understanding of the precursors and characterisation of enzymes involved in the pathways leading to the formation of flavours in fruit is essential for the production of natural flavours. Plant derived flavour 20 components alone represent a world-wide market of 1.5 billion dollars. Presently, the main ways to produce plant flavour compounds is by the synthetic route. Synthetic organic chemicals constitute more than 80-90% (by weight and value) of the raw materials used in flavour and fragrance 25 formulations. Problems often exist concerning production. Extraction from intact plants and conventional fermentation are currently providing alternative routes for the commercial production of flavour and aroma chemicals. However, the demand for natural flavours by the consumer has been steadily 30 increasing, and often demand outstrips supply. In many cases sought after flavour compounds preclude isolation. Unlike other fruit flavours, no single chemical or class of chemicals in particular are associated with strawberry flavour. To date no strawberry fruit specific aminotransferase, 35 pyruvate decarboxylase, thiolase, alcohol dehydrogenase, or acyl-transferase enzymes at the route of this invention, involved directly or in providing precursors for ester formation, hence flavour compounds, has been isolated and

characterized. The genes and their peptides at the route of this invention are involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit and can therefore provide a novel method for the *in-vivo* and *in-vitro* biotechnology production of bio-flavours, natural flavour chemicals by recombinant means.

Summary of invention

The object of the present invention is to disclose the genes/peptides involved in the process of volatile ester production, particularly aliphatic esters although not exclusively so, hence fruit flavour and aroma. This is the first time to our knowledge, that the genes/peptides sequences from an entire pathway to the production of volatile esters in fruit, in particularly strawberry, have been disclosed. The nucleotide and polypeptide sequences described in this application can serve as a tool for a vast number of applications related to ester formation and the biotechnological engineering of natural and artificial fruit flavours for the food industry. The invention is based on the identification of genes which encode proteins central to the pathway leading to volatile ester formation in fruit. DNA sequences which encode these proteins have been cloned and characterised. The nucleic acid/peptide sequences may be used in expression systems, for industrial application and /or to modify plants with the goal to produce natural and/or synthetic flavours.

Definitions of Terms

'Nucleic acid' sequence as used herein refers to an oligonucleotide, nucleotide or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represents the sense or antisense strand. Similarly, 'amino acid sequence' as used herein refers to peptide or protein sequence.

The term 'agonist', as used herein, refers to a molecule which, when bound to a polypeptide causes a change in the polypeptide which modifies the activity of the polypeptide. Agonist may include proteins, nucleic acids, 5 carbohydrates, or any other molecule which binds to the polypeptide.

The term 'antagonist' or 'inhibitor' as used herein, refer to a molecule which when bound to a polypeptide, blocks or modulates the biological or immunological 10 activity of the polypeptide. Antagonists or inhibitors may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to the polypeptide.

'Stringency' typically occurs in the range from about $T_m - 5^\circ\text{C}$ (5°C below the T_m of the probe) to about 20°C to 15 25°C below T_m . As will be understood by those skilled in the art, a stringent hybridisation can be used to identify or detect identical polynucleotide sequences or to identify or detect similar or related polynucleotide sequences.

The term 'hybridisation' as used herein shall 20 include 'any process' by which a strand of nucleic acid joins with a complementary strand through base pairing' (Coombs J, (1994) Directory of biotechnology, Stockton press, New York NY).

A 'deletion' is defined as a change in either 25 nucleotide or amino acid sequence in which one or more nucleotides or amino acid residues, respectively, are absent.

An 'insertion' or 'addition' is that change in nucleotide or amino acid sequence which has resulted in the 30 addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring polypeptide(s).

A 'substitution' results from the replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively.

As used herein, the term 'substantially purified' refers to molecules, either nucleic or amino acid sequences, that are removed from their natural environment, isolated or

separated, and that are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated. The definition 'substantially homologous' means that a particular subject 5 sequence, for example a mutant sequence, varies from the reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an adverse functional dissimilarity between the reference and the subject sequence.

10 A 'variant' of a polypeptide as outlined in the first and second aspects of this invention is defined as an amino acid sequence that is different by one or more amino acid 'substitutions'. A variant may have 'conservative' changes, wherein a substituted amino acid has similar 15 structural or chemical properties eg replacement of leucine with isoleucine. More rarely a variant may have 'non-conservative' changes, eg replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining 20 which and how many amino acid residues may be substituted, inserted or deleted, without abolishing biological or immunological activity may be found using computer programmes well known in the art, for example, DNASTar software.

The term 'biologically active' refers to a polypeptide as outlined in the first and second aspects of the invention, having structural, regulatory, or biochemical function of their naturally occurring counterparts. Likewise, 'immunologically active' defines the capability of the natural, recombinant or synthetic polypeptide, as outlined 30 in the first and second aspects of the invention, or any oligopeptide thereof, to induce a specific immune response in an appropriate animal or cells and to bind with specific antibodies.

The term 'natural products' are those products that 35 are obtained directly from plants and sometimes from animal sources by physical procedures. 'Nature identical compounds' are produced synthetically but are chemically identical to their 'natural counterparts'. Artificial flavour substances

are compounds that have not been identified in plant or animal products for human consumption. 'Nature-identical' aroma substances are with few exceptions the only synthetic compounds used in flavours in addition to 'natural products'.
5

The definition 'host cell' refers to a cell in which an alien process is executed by bio-interaction, irrespective of the cell belongs to a unicellular, multicellular, a differentiated organism or to an artificial
10 cell, cell culture or protoplast. The definition 'host cell' in the context of this invention is to encompass the definition 'plant cell'.
15

'Plant cell' by definition is meant by any self-propagating cell bounded by a semi permeable membrane and
15 containing one or more plastids. Such a cell requires a cell wall if further propagation is required. 'Plant cell', as used herein, includes without limitation, seeds, suspension cultures, embryos, meristematic regions, callous tissues, protoplasts, leaves, roots, shoots, gametophytes, sporophytes,
20 pollen and microspores.

With the definition 'transformed cell' or 'transgenic cell' is meant a cell (or ancestor of said cell) into which by means of recombinant DNA techniques, DNA encoding the target polypeptide can be introduced.

25 The definition 'micro-organisms' refers to microscopic organisms, such as Archaea, Bacteria, Cyanobacteria, Microalgae, Fungi, Yeast, Viruses, Protozoa, Rotifers, Nematodes, Micro-Crustaceans, Micro-Molluscs, Micro-Shellfish, Micro-insects etc.

30 The definition 'plant(s)' refers to eukaryotic, autotrophic organisms. They are characterised by direct usage of solar energy for their primary metabolism, their permanent cell wall and in case of multicellular individuals their open unlimited growth. In case of heterotrophic
35 plants, the organisms are in an evolutionary context essentially derived from autotrophic plants in their structure and metabolism.

'Dicotyledons' (and all scientific equivalents referring to the same group of plants) form one of the two divisions of the flowering plants or angiospermae in which the embryo has two or more free or fused cotyledons.

5 'Monocotyledons' (and all scientific equivalents referring to the same group of plants) form one of the two divisions of the flowering plants or angiospermae in which the embryo has one cotyledon.

'Angiospermae' or flowering plants are seed plants 10 characterised by flowers as specialised organs of plant reproduction and by carpels covering the ovaries. Gymnospermae are seed plants characterised by strobili as specialised organs for plant reproduction and by naked sporophylls bearing the male or female reproductive organs. 'Ornamental' plants are plants that are primarily in cultivation for 15 their habitus, special shape, (flower, foliage or otherwise) colour or other characteristics which contribute to human well being indoor as cut flowers or pot plants or outdoors in the man made landscape. 'Vegetables' are plants that are 20 purposely bred or selected for human consumption of foliage, tubers, stems, fruits, flowers or parts of them and that need an intensive cultivation regime. 'Arable crops' are purposely bred or selected for human objectivity's (ranging 25 from direct or indirect consumption, feed or industrial applications such as fibres).

The definition 'process' is the development of, a method(ology) concerning, the progress of a series of activities in a certain context. 'Biological' process is a process based on metabolic activity in organisms, or essentially 30 derived from that by biochemical, biophysical, physiological, ecological or genetic means. 'Industrial' process is an economically feasible process with high added value, aiming at bulk production.

The definition 'homology' refers to the basic 35 similarity of a particular structure in different organisms, usually as result from the descent from a common ancestor.

The definition 'promoter' is intended as a nucleotide sequence sufficient to direct transcription. Also

included are those promoter elements which are sufficient to render tissue-specific gene expression; such elements may be located in the 5' or 3' regions of the native gene.

5 The definition 'operably linked' is meant that a gene and a regulatory sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (for example, transcriptional activator proteins) are bound to the regulatory sequence(s).

10 The definition 'fruit' (botanically) is the ripened ovary of a plant and its contents. The definition 'fruit' (agronomically linguistically) is the ripened ovary and its contents together with any structure with which they are combined, as in case of strawberry or apple, the receptacle.

15 The definition 'climacteric' is pointing at the phase of increased respiration found at fruit ripening and at senescence. The definition 'non-climacteric' is pointing at no such phase being present.

20 The definition 'antisense' RNA is an RNA sequence which is complementary to a sequence of bases in the corresponding mRNA: complementary in the sense that each base (or majority of bases) in the antisense strand (read in the 5' to 3' sense) is capable of pairing with the corresponding base (G with C, A with U), in the mRNA sequence read in the 5' to 3' sense.

25 The definition 'sense' RNA is an RNA sequence which is substantially homologous to at least part of the corresponding mRNA sequence.

Brief description of drawings

30 The present invention may be more fully understood by reference to the following description, when read together with the accompanying drawings:

Figure 1. The proposed biosynthetic pathway for the production of esters in fruits.

Figure 2. A GCMS chromatogram of a mature strawberry fruit of the cultivar *Elsanta*. The analysis was done using the headspace method. Esters are marked with dots.

5 Figure 3. The expression pattern of strawberry alcohol acyl transferase cDNA (SLE27, SEQ ID NO:1A) in various tissues of the cultivar *Elsanta* by Northern blot analysis. The Northern blot was hybridised with a full length cDNA fragment of SEQID:1A (SLE27).

10 Figure 4. The expression pattern of a lemon alcohol acyl transferase (CLF26, SEQ ID NO:2A) in various tissues of the lemon cultivar *Mayer* by Northern blot analysis. The Northern blot was hybridised with a full length cDNA fragment of SEQ ID 2A (CLF26).

15 Figure 5. Expression analysis in various tissues of the strawberry cultivar *Elsanta* by Northern blots analysis. The Northern blots where hybridised with full length cDNA fragments corresponding to (a) a thiolase SEQ ID 4A (SLG150) (b) a pyruvate decarboxylase SEQ ID 5A (SLH51) and (c) an 20 alcohol dehydrogenase SEQ ID 6A (SLB39).

Figure 6. Strawberry genomic Southern analysis. 10 μ g DNA was digested with restriction enzymes HindIII, EcoRI, XbaI and XhoI, separated on an agarose gel and blotted onto a nylon filter. The DNA was hybridised with a full length 32 P-labelled strawberry alcohol acyl transferase cDNA (SLE27) probe, corresponding to SEQ ID 1A and autoradiographed.

Figure 7. A scheme of the pRSET B (Invitrogen) vector used for the expression of the strawberry alcohol acyl transferase (SLE27, SEQ ID NO: 1A) in *E.coli*.

30 Figure 8. Shows acetic acid butyl ester formation from acetyl-CoA and 1-butanol by (A) 25 μ l of protein elute from *E. coli* expressing the strawberry alcohol acyl transferase (SLE27, SEQ ID NO:1A) (B) 25 μ l of inactivated protein elute

from *E. coli* expressing the strawberry alcohol acyl transferase (SLE27, SEQ ID NO:1A) (C) 25 µl of protein extract from *E. coli* expressing the empty vector cassette.

Figure 9. Western blot loaded with protein corresponding to 5 four different treatments carried out on the pellet, supernatant and elute from a Ni-NTA column after expression of strawberry alcohol acyl transferase (SLE 27, SEQ ID NO:1A) in *E. coli*. As a control for the experiment the Green Fluorescent Protein (GFP) was partially purified in the same 10 way and the elute from one treatment was loaded on the gel. The blot was hybridised with a commercial antibody (Clon- tech) raised against an epitope fused at the N- terminus of the recombinant protein.

Figure 10. Gene expression profiles of strawberry alcohol 15 acyl transferase (SLE 27, SEQ ID NO: 1A)), alcohol dehydro- genase (SLF138, SEQ ID NO: 11A), pyruvate decarboxylase (SLH 51, SEQ ID NO: 5A), alcohol dehydrogenase (SLG16, SEQ ID NO:12A) and amino transferase (SLF96, SEQ ID NO: 3A). Ex- 20 pression ratios were monitored during fruit development and ripening in the red stage vs green fruit and red fruit v.s turning fruit.

Detailed description of the invention

According to a first aspect the invention provides an insolated DNA sequence encoding

25 (a) a polypeptide having an amino acid sequence as shown in SEQ ID NO: 1B or SEQ ID NO: 2B, or

(b) a polypeptide having at least 30% homology with the amino acid sequence SEQ ID NO: 1B or at least 40% homology with the amino acid sequence 30 SEQ ID NO: 2B, or

(c) a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has alcohol acyl transferase activity and is involved in the biosynthetic pathway for ali-

phatic and /or aromatic ester production in fruit.

Preferably, the DNA sequence encodes a polypeptide having at least 50% homology, more preferably at least 70% homology, with any of the amino acid sequences SEQ ID NO: 1B or SEQ ID NO: 2B, or a fragment thereof.

With reference to the nucleic acid sequence the invention provides an isolated DNA sequence having a nucleic acid sequence

- 10 (a) as shown in SEQ ID NO: 1A or SEQ ID NO: 2A which encodes a polypeptide having alcohol acyl transferase activity and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, or
- 15 (b) complementary to SEQ ID NO: 1A or SEQ ID NO: 2A, or
- (c) which has at least 25% homology with any of the sequences of (a) or (b), or
- (d) which is capable of hybridising under stringent 20 conditions to any of the sequences (a)-(c).

Preferably the DNA sequence has a nucleic acid sequence

- (c) which has at least 40% homology, more preferably at least 60% homology, with any of the sequences of (a) or (b), or
- 25 (d) which is capable of hybridising under stringent conditions to said sequence (c).

According to a second aspect the invention provides a purified and isolated polypeptide,

- 30 (a) having an amino acid sequence as shown in SEQ ID NO: 1B or SEQ ID NO: 2B, or
- (b) having at least 30% homology with the amino acid sequence SEQ ID NO: 1B or at least 40% homology with the amino acid sequence SEQ ID NO: 2B, or
- 35 (c) being a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has alcohol acyl transferase activity and is

involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

5 Preferably, the polypeptide has at least 50% homology, more preferably at least 70% homology, with any of the amino acid sequences SEQ ID NO: 1B or SEQ ID NO: 2B, or a fragment thereof.

It may be advantageous to produce nucleotide sequences according to the first aspect of the invention as 10 defined above or derivatives thereof possessing a substantially different codon usage. It is known by those skilled in the art that as a result of degeneracy of the genetic code, a multitude of gene sequences, some bearing minimal homology 15 to the nucleotide sequences of any known and any naturally occurring genes may be produced. The invention contemplates each and every possible variation of the nucleotide sequences that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied 20 to the nucleotide sequence of naturally occurring gene sequences, and all such variations are to be considered as being specifically disclosed. In addition the nucleotide sequences of the invention may be used in molecular biology techniques that have not been developed, providing the new 25 techniques rely on properties of nucleotide sequences that are currently known, including but are not limited to such properties such as the triplet genetic code and specific base pair interactions.

Altered nucleic acid sequences of this invention 30 include deletions, insertions, substitutions of different nucleotides resulting in the polynucleotides that encode the same or are functionally equivalent. Deliberate amino acid substitution may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, and/or the 35 amphipathetic nature of the residues as long as the biological activity of the polypeptide is retained. Included in the scope of the present invention are alleles of the polypeptides according to the second aspect of the invention

as defined above. As used herein, an 'allele' or 'allelic sequence' is an alternative form of the polypeptides described above. Alleles result from a mutation, eg a change in the nucleic acid sequence, and generally produce altered 5 mRNA or polypeptide whose structure or function may or may not be altered. Any given polypeptide may have none, or more allelic forms. Common allelic changes that give rise to alleles are generally ascribed to natural deletions, additions or substitutions of amino acids. Each of these types of 10 changes may occur alone, or in combination with the others, one or more times in a given sequence.

It is envisaged that the polynucleotide sequences of the present invention can be used as probes for the isolation of similar sequences from the strawberry genome 15 and lemon genome. By using as a probe a cDNA of the gene sequences of the invention it would be possible by those skilled in the art to obtain comparable gene sequences from various tissues, particularly fruit tissues, from climacteric and non climacteric plants. One aspect of the invention 20 is to provide for hybridisation or PCR probes which are capable of detecting polynucleotide sequences, including genomic sequence(s), encoding the polypeptides of the invention, or closely related molecules. The specificity of the probe, whether it is made from a highly specific region, 25 eg 10 unique nucleotides in the 5' regulatory region, or a less specific region, e.g. in the 3' region, and the stringency of the hybridisation or amplification (maximal, high, intermediate, low) will determine whether the probe identifies only naturally occurring sequence(s) encoding the 30 polypeptide, allele's or related sequences.

Probes may also be used for the detection of related sequences and preferably contain at least 50% of any of the nucleotides from any one of the gene encoding sequences according to the present invention. For gene sequences 35 of the present invention, the hybridisation probes may be derived from the nucleotide sequence, or from genomic sequence including promoter, enhancer element and introns. Hybridisation probes may be labelled by a variety of rep-

ter groups, including radionuclides such as ^{32}P or ^{35}S , or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Other means for producing specific hybridisation probes for encoding gene sequences of the invention include the cloning of nucleic acid sequences into suitable vectors for the production of mRNA probes. Such vector are known in the art and are commercially available and may be used to synthesise RNA probes *in-vitro* by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA polymerase and the appropriately radioactively labeled nucleotides.

According to a third aspect the invention provides a recombinant expression vector comprising a coding sequence which is operably linked to a promoter sequence capable of directing expression of said coding sequence in a host cell for said vector, and a transcription termination sequence, in which the coding sequence is a DNA sequence according to the invention.

In a further embodiment the invention provides a replicative cloning vector comprising an isolated DNA sequence of the invention and a replicon operative in a host cell for said vector.

Methods which are well known to those skilled in the art can be used to construct expression vectors containing the gene coding sequence of the invention, and appropriate transcriptional and translational controls. These methods include *in-vitro* recombinant techniques. Such techniques are described in Sambrook et al., 1989. Molecular cloning a laboratory manual, cold spring Harbour press, Plain view, NY and Ausubel FM et al., (1989) Current protocols in molecular biology, John Wiley and Sons, New York, NY.

In order to express a biologically active polypeptide, the nucleotide sequence encoding the polypeptide, or the functional equivalents thereof, fragments of the polypeptide, is inserted into the appropriate expression vector (i.e. a vector that contains the necessary elements for the transcription or translation of the inserted coding

sequence). Specific initiation signals may also be required for efficient translation of the polypeptides of the invention. These signals include the ATG initiation codon and adjacent sequences. In cases where the polypeptides, their 5 initiation codons and upstream sequences are inserted into the appropriate expression vector, no additional translational control systems including the ATG initiation codon must be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure transcription of the 10 entire insert. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (Scharf D et al (1994) *Results Prob Cell Differ* 20: 125-15 62; Bittner et al. (1987) *Methods in Enzymol* 153: 516-544).

In addition a host strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not 20 limited to, acylation, carboxylation, glycosylation, phosphorylation and lipidation. Post translation processing which cleaves a 'prepro' form of the protein may also be important for correct insertion, folding and/or function. Different host cells which have the correct cellular machinery and characteristic mechanisms for such post-translational 25 activities maybe chosen to ensure correct modification and processing of the introduced, foreign protein.

The nucleotide sequences of the present invention can be engineered in order to alter the coding sequence for 30 a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example mutations may be introduced using techniques which are well known in the state of the art, e.g. site directed mutagenesis to insert 35 new restriction sites, to alter glycosylation patterns, to change codon usage, to produce splice variants etc. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular procaroytic or eukaroytic

expression host in accordance with the frequency with which particular codons are utilised by the host (Murray E et al. (1989) *Nuc Acids Res* 17: 477-508). Other reasons for substantially altering the nucleotide sequence(s) of the 5 invention and their derivatives, without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half life, than transcripts produced from naturally occurring sequences.

10 In developing the expression cassette, the various fragments comprising the regulatory regions and open reading frame may be subjected to different processing conditions, such as ligation, restriction enzyme digestion, resection, *in-vitro* mutagenesis, primer repair, use of linkers and 15 adapters and the like. Thus, nucleotide transitions, transversions, insertions, deletions and the like, may be performed on the DNA which is employed in the regulatory regions and/or open reading frame. The expression cassette may be wholly or partially derived from natural sources endogenous 20 to the host cell. Furthermore, the various DNA constructs (DNA sequences, vectors, plasmids, expression cassettes) of the invention are isolated and/or purified, or synthesised and thus are not naturally occurring. The invention further 25 contemplates the use of yet undescribed biological and non biological based expression systems and novel host(s) systems that can be can be utilised to contain and express the gene coding sequence(s) of the invention.

'Antisense' or 'partial sense' or other techniques may also be used to reduce the expression of genes in the 30 pathway leading to the production of flavour volatile esters. Full length 'sense' techniques may be designed to increase or reduce the expression of genes in the pathway leading to the production of flavour volatiles. The 'anti-sense' or 'partial sense' molecules may be designed to block 35 translation of mRNA by preventing the transcript from binding to the ribosomes. Ribozymes are enzymatic RNA molecules capable of catalysing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-

specific hybridisation of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyse endonucleolytic cleavage of gene sequences of the invention. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridisation with complementary oligonucleotides using ribonuclease protection assays.

'Antisense' molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in-vitro* and *in-vivo* transcription of DNA sequences of the invention. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 and SP6. Alternatively, antisense cDNA constructs that synthesise antisense RNA constitutively or inducibly can be introduced into cell lines, cells or tissues. RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences, at the 5' and/or 3' end of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule.

A variety of vector/host expression systems can be utilised to contain and express the gene coding sequences of the invention. These include and are not limited to micro-

organisms such as bacteria (e.g. *E. coli*, *B. subtilis*, *Streptomyces*, *Pseudomonads*) transformed with recombinant bacteriophage, plasmid or cosmid DNA expression systems, yeast (e.g. *S. cerevisiae*, *Kluyveromyces lactis*, *Pichia pastoris*,
5 *Hansenula polymorpha*, *Schizosacch. Pombe*, *Yarrowia*) transformed with yeast expression vectors; filamentous fungi (*Aspergillus nidulans*, *Aspergillus orizae*, *Aspergillus niger*) transformed with filamentous fungi expression vectors, insect cell systems transfected with virus expression
10 vectors (e.g. baculovirus, adenovirus, herpes or vaccinia viruses); plant cell systems transfected with virus expression vectors (e.g. cauliflower mosaic virus, CaMV, tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (e.g. Ti or Pbr322 plasmid); or mammalian cell
15 systems (Chinese hamster ovary (CHO), baby hamster kidney (BHK), Hybridoma's, including cell lines of mouse, monkey, human and the like.

In the case of plant expression vectors, the expression of a sequence (s) of the invention may be driven
20 by a number of previously defined promoters, including inducible and developmentally regulated promoters. The invention further contemplates the use of the individual promoters of the polynucleotide sequence(s) of the present invention for this purpose. In particular an alcohol acyl
25 transferase promoter(s) pertaining to SEQ ID IA and SEQ ID 2A or any promoters particularly responsive to ripening events may be used to drive the tissue specific expression of the target genes. In addition, viral promoters such as the 35S and the 19S promoters of CaMV (Brisson et al., 1984;
30 Nature, 310: 511 -514) may be used alone or in combination with the omega leader sequence from TMV (Takamatsu et al. (1987); EMBO J 6:307-311). Promoters or enhancers derived from the genomes of plant cells, tissue specific promoters i.e fruit specific promoters, Fbp7 (Columbo et al. 1997;
35 Plant Cell 9; 703-715), 2A11 promoter (Pear et al., 1989, Plant Molecular Biology, 13:639-651), small subunit of Rubisco (Corruzzzi et al., 1984; EMBO J 3:16; Broglie et al., 1984 Science 224:838-843) or timing specific promoters such

as ripening specific promoters (the E8 promoter, Diekman and Fisher, 1988, *EMBO J*, 7:3315-3320) may be used. Suitable terminator sequences include that of the *agrobacterium tumefaciens* nopaline synthase gene (Nos 3' end), the tobacco 5 ribulose bisphosphate carboxylase small subunit termination region; and other 3' regions known to those skilled in the art. These constructs can be introduced into plant cells by direct DNA transformation, or pathogen mediated transfection. For reviews of such techniques, see Hobbs S or Murry 10 LE, in McGraw Hill yearbook of Science and technology (1992), Mc Graw Hill NY, PP 191-196 or Weissbach and Weissbach (1988) Methods for Plant Molecular Biology, Academic Press, New York, NY, pp421-463, Fillatti et al., Biotechnology, 5: 726-730. Manipulation of DNA sequences in plant 15 cells may be carried out using the Cre/lox site specific recombination system as outlined in patent application WO91-09957.

According to a fourth aspect the invention provides a method for regulating aliphatic and/or aromatic ester 20 formation in fruit, comprising inserting into the genome of a fruit-producing plant one or more copies of one or more DNA sequences of the invention.

The activity of genes according to the present invention involved in the biosynthetic pathway leading to 25 volatile ester formation, hence flavour, may be either increased or reduced depending on the characteristics desired for the modified plant part. The gene sequence may be the in the same 'sense' or 'antisense' orientation as the endogenous target gene. Methods well known to those skilled 30 in the art can be used to construct recombinant vectors which will express 'sense' or 'antisense' polynucleotides encoding the gene sequences of the present invention. Such technology is now well known in the art. In the case of the 'plant cell' officiating as 'host cell', the target 'plant 35 cell' may be part of a whole plant or may be an isolated cell or part of a tissue which may be regenerated into a whole plant. The target plant may be selected from any monocotyledonous or dicotyledonous plant species. Suitable

plants include any fruit bearing plant such as strawberry, citrus (lemon), banana, apple, pear, melon, sweet pepper, peach or mango. Other suitable plant hosts include vegetable, ornamental (to include sunflower) and arable crops (to 5 include soybean, sunflower, corn, peanut, maize, wheat, cotton, safflower, and rapeseed). For any particular plant cell, the gene sequences, cDNA, genomic DNA, or synthetic polynucleotide, used in the transformation vector construct, may be derived from the same plant species, or may be 10 derived from other plant species (as there will be sufficient homology to allow modification of related enzyme gene function). The procedure or method for preparing a transformant can be performed according to the conventional technique used in the fields of molecular biology, biotechnology 15 and genetic engineering.

According to a fifth aspect the invention provides a plant and propagating material thereof which contains in its genome a DNA sequence of the invention or a vector as defined above.

20 In a preferred embodiment the invention provides a genetically modified strawberry or lemon plant and propagating material derived therefrom which has a genome comprising an expression vector for over-expression or down-regulation of an endogenous strawberry or lemon plant gene 25 counterpart of any of the DNA sequences of the invention.

In mammalian cells a number of viral based systems may be utilised. In cases where the adenovirus is used as an expression vector, a gene coding sequence(s) of the invention may be ligated into the adenovirus transcription/translation complex consisting of the late promoter and tripartite 30 leader sequence. Insertion in a non essential E1 or E3 region of the viral genome will result in a variable virus capable of expressing the gene sequence(s) in infected host cells (Logan and Shark (1984) *Proc Natl Acad. Sci* 81:3655-35 59) In addition transcription enhancers, such as the *Rous sarcoma virus* (RSV) enhancer, may be used to increase expression in mammalian host cells.

Furthermore insect cells such as silkworm cells or larvae themselves may be used as a host. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The gene coding sequence(s) of the invention may be cloned into the nonessential region of the virus, such as the polyhedrin gene, and placed under control of a polyhedrin promoter. Successful insertion of the sequence(s) will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses are then used to infect *S frugiperda* cells or *Trichoplusia* larvae in which the gene sequence or sequences are expressed (Smith et al. (1993) *J Virol* 46:584; Engelhard et al. (1994) *Proc. Natl acad Sci*, 91: 3224-7).

The control elements or regulatory sequences of the described systems vary in their strength and specificities and are those nontranslated regions of the vector, enhancers, promoters and 3' untranslated regions, which interact with host cellular proteins to carry out transcription and translation. Dependent on the vector system and host utilised, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript [®] phagemid (Strategene, La Jolla) or pSPORT (Gibco BRL) and ptrp-lac hybrids and the like may be used. Other conventionally used promoters are trc and trc promoters. The baculovirus polyhedrin promoter may be used for insect cells. As a promoter for filamentous organisms, for example, amalyse, trp C, and the like may be used. As a promoter for yeast for example glyceraldehyde-3-phosphate dehydrogenase may be used. As a promoter for animal cell hosts, viral promoters such as the SV40 early promoter, SV40 late promoter and the like may be used. In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as the alpha factor,

alcohol oxidase and PGH may be used. For review, see Grant et al., 1987 *Methods in Enzymology* 153: 516-544.

For long term, high yield production of recombinant proteins, stable expression is preferred. For example 5 cell lines which stably express the polypeptides of the invention may be transformed using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 10 one to two days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clumps of stable 15 transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

Although the presence/absence of the marker gene expression suggests that the gene of interest is also present, its presence and expression must be confirmed. For 20 example, if the gene sequence(s) of the invention are inserted within a marker gene sequence, recombinant cells can be identified by the absence of gene function. Alternatively a marker gene can be placed in tandem with the gene sequence of interest, under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem 25 gene as well.

Any number of selection systems may be used to recover the transformed cell lines. Anti-metabolite, antibiotic 30 or herbicide resistance can be used as the basis of selection; for example, dhfr which confers resistance to methotrexate (Wiger M. et al. (1980) *Proc Natl Acad Sci* 77: 3567-70; npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin F. et al. (1981) *J 35 Mol Biol* 150: 1-14) and als and pat, which confer resistance to chlorsulfuron and phosphinotricin acyl transferase, respectively (Murry supra). Additional selectable genes have been transcribed, for example, trpB, which allows cells

utilise indole in place of tryptophan, or His D, which allows cells to utilise histinol in place of histidine (Hartman SC and RC Mulligan (1988) *Proc Natl Acad Sci* 85: 8047-51). Alternatively one could use visible markers such 5 as anthocyanidins, β -glucuronidase and its substrates, GUS, GFP and variants, and luciferase and its substrate, luciferin, which are widely used to identify transformants, but also to quantify the amount of stable protein expression attributable to a specific vector system (Rhodes CA et al. 10 (1995) *Methods Mol Biol* 55: 121-131).

Host cells transformed with a nucleotide sequence(s) of the invention, may be cultured under conditions suitable for expression and recovery of the encoded protein from cell culture. The protein produced by recombinant cells 15 may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those skilled in the art, expression vectors containing polynucleotides sequences of the invention, can be designed with signal sequences for direct secretion of the protein 20 product, through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may join the gene sequences of the invention, to a nucleotide sequence encoding a polypeptide domain, which will facilitate purification of 25 soluble proteins (Kroll DJ et al. (1993). *DNA Cell Biol* 12: 441-53).

According to a sixth aspect the invention provides a method for producing aromatic and/or aliphatic esters in microorganisms, plant cells or plants comprising inserting 30 into the genome of the microorganism or plant one or more copies of DNA sequences of the invention, and feeding an alcohol and an acyl-CoA to the microorganism or plant (cell).

Alternatively, host cells which contain the gene sequences or polypeptides thereof according to the invention 35 may be identified by a variety of procedures known to those skilled in the art. These procedures include, but are not limited to DNA-DNA, DNA-RNA hybridisation, amplification using probes (portions or fragments of polynucleotides),

protein bioassay or immunoassay techniques which include membrane, solution or chip based technologies for the detection and /or quantification of the nucleic acid or protein.

5 Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the gene sequences of the invention to detect transformants containing DNA or RNA encoding the polypeptides. As used herein 'oligonucleotides' or 'oligomers' refer to nucleic acid
10 sequence of at least 10 nucleotides and as many as about 60 nucleotides, preferably about 15-30 nucleotides, and more preferably about 20-25 nucleotides which can be used as a probe or amplimer. Polynucleotide sequences according to the invention encoding alcohol acyl transferase polypeptides may
15 be used to detect volatile ester (aliphatic and aromatic) formation in fruits. PCR as described in US patent Nos 4,683,195 and 4,965,188, provides additional uses for oligonucleotides based on the alcohol acyl transferase sequence. Such oligomers are chemically synthesised, but
20 they may be generated enzymatically or produced from a recombinant source. Oligomers generally comprise two nucleotide sequences, one with sense orientation (5'-3') and one with antisense (3'-5'), employed under less stringent conditions for detection and /or quantitation of closely
25 related DNA or RNA sequences. For example, polynucleotide sequences encoding the alcohol acyl transferase protein may be used in the hybridisation or PCR assay of plant tissues, more specifically fruit tissues, to detect alcohol acyl transferase protein presence. The form of such quantitative
30 methods may include, Southern or Northern analysis, dot/slot blot-or-other-membrane based technologies; PCR technologies such as DNA Chip, Taqman and ELISA Technologies. All of these technologies are well known in the art and are the basis of many commercially owned diagnostic kits. The
35 polynucleotide sequences according to the present invention herein provide the basis for assays that detect signal transduction events associated with volatile ester production, aliphatic and aromatic, hence flavour formation.

A variety of protocols for detecting and measuring expression of the polypeptides of the invention, using either polyclonal or monoclonal antibodies specific for the protein are well known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). These methods are described, among other places, in Hampton R et al. (1990), Serological methods, a laboratory manual, APS Press, St Paul MN) and Maddox DE et al. (1993, *J Exp Med* 158:1211).

A variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labelled hybridisation or PCR probes for detecting sequences related to polynucleotides as outlined in the first and second aspects of the invention, include oligolabeling, nick translation, end labelling of PCR amplification using a labelled nucleotide. Alternatively, the gene sequence or sequences of the invention, or any portion of it, may be cloned into a vector for the production of the mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesise RNA probes in-vitro by addition of an appropriate RNA polymerase, such as T7, T3, or SP6 and labelled nucleotides. A number of companies such as Pharmacia Biotech (Piscataway NJ), promega (Madisson WI), and US Biochemical Corp (Cleveland OH) supply commercial kits and protocols for these procedures. Suitable reporter molecules or labels include fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles and the like.

According to another aspect of the invention, natural, modified or recombinant polynucleotide sequences according to the invention may be ligated to a heterologous sequence to encode a fusion protein. A fusion protein may be engineered with one or more additional domains added to facilitate protein purification (i.e. a cleavage site located between the sequence of the polypeptide and the heterologous protein sequence) so that the polypeptide of

the invention may be purified away from the heterologous moiety. The invention further contemplates the creation of fusion proteins, chosen from the group of sequences of the invention, degenerate variants thereof, functional equivalents thereof, pertaining to 'fruit' of strawberry and lemon, or gene sequences with substantial homology to the sequences of the invention from other 'fruit' tissues concerned with the pathway for the formation of aliphatic or aromatic esters, hence flavour. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilised metals, protein A domains that allow purification on immobilised immunoglobin, and the domain utilised in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, WA). The introduction of a cleavable linker such as factor XA, thrombin or enterokinase (Invitrogen, San Diego CA) between the purification domain and the polypeptide is useful to facilitate purification. One such expression vector provides for the expression of a fusion protein comprising gene sequence(s) which encode polypeptides of the invention, and contains nucleic acid encoding six histidine residues followed by thioredoxin and the enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilised metal ion affinity chromatography as described in Porath *et al.*, (1992) Protein Expression and Purification 3:263-281) while the enterokinase cleavage site provides a means for purifying the polypeptide from the fusion protein.

According to a seventh aspect the invention provides an isolated DNA sequence encoding

- ... (a) a polypeptide having an amino acid sequence as shown in SEQ ID NO: 3B, or
- (b) a polypeptide having at least 80% homology with the amino acid sequence SEQ ID NO: 3B, or
- (c) a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has aminotransferase activity and is involved

in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

In particular the invention provides an isolated DNA sequence having a nucleic acid sequence

5 (a) as shown in SEQ ID NO: 3A which encodes a polypeptide having aminotransferase activity and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, or

10 (b) complementary to SEQ ID NO: 3A, or

(c) which has at least 70 % homology with any of the sequences of (a) or (b), or

(d) which is capable of hybridising under stringent conditions to any of the sequences (a) - (c).

15 Further the invention provides a purified and isolated polypeptide,

(a) having an amino acid sequence as shown in SEQ ID NO: 3B, or

(b) having at least 80% homology with the amino acid sequence SEQ ID NO: 3B, or

20 (c) being a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has aminotransferase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

25

According to an eighth aspect the invention provides an isolated DNA sequence encoding

(a) a polypeptide having an amino acid sequence as shown in SEQ ID NO: 4B, or

30 (b) a polypeptide having at least 90% homology with the amino acid sequence SEQ ID NO: 4B, or

(c) a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has thiolase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

35

In particular the invention provides an isolated DNA sequence having a nucleic acid sequence

5 (a) as shown in SEQ ID NO: 4A which encodes a polypeptide having thiolase activity and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, or

10 (b) complementary to SEQ ID NO: 4A, or

(c) which has at least 75 % homology with any of the sequences of (a) or (b), or

15 (d) which is capable of hybridising under stringent conditions to any of the sequences (a) - (c).

Further the invention provides a purified and isolated polypeptide,

20 (a) having an amino acid sequence as shown in SEQ ID NO: 4B, or

25 (b) having at least 90% homology with the amino acid sequence SEQ ID NO: 4B, or

(c) being a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has thiolase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

According to a ninth aspect the invention provides an isolated DNA sequence encoding

30 (a) a polypeptide having an amino acid sequence as shown in SEQ ID NO: 5B, or

(b) a polypeptide having at least 90% homology with the amino acid sequence SEQ ID NO: 5B, or

(c) a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has pyruvate decarboxylase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

35 In particular the invention provides an isolated DNA sequence having a nucleic acid sequence

(a) as shown in SEQ ID NO: 5A which encodes a polypeptide having pyruvate decarboxylase activity and being involved in the biosynthetic pathway

for aliphatic and/or aromatic ester production in fruit, or

- (b) complementary to SEQ ID NO: 5A, or
- (c) which has at least 75 % homology with any of the sequences of (a) or (b), or
- (d) which is capable of hybridising under stringent conditions to any of the sequences (a) - (c).

Further the invention provides a purified and isolated polypeptide,

- 10 (a) having an amino acid sequence as shown in SEQ ID NO: 5B, or
- (b) having at least 90% homology with the amino acid sequence SEQ ID NO: 5B, or
- 15 (c) being a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has pyruvate decarboxylase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

According to a tenth aspect the invention provides 20 an isolated DNA sequence encoding

- (a) a polypeptide having an amino acid sequence as shown in SEQ ID NO: 6B, or
- (b) a polypeptide having at least 75% homology with the amino acid sequence SEQ ID NO: 6B, or
- 25 (c) a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has alcohol dehydrogenase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

In particular the invention provides an isolated DNA sequence having a nucleic acid sequence

- 35 (a) as shown in SEQ ID NO: 6A which encodes a polypeptide having alcohol dehydrogenase activity and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, or
- (b) complementary to SEQ ID NO: 6A, or

- (c) which has at least 65 % homology with any of the sequences of (a) or (b), or
- (d) which is capable of hybridising under stringent conditions to any of the sequences (a) - (c).

5 Further the invention provides a purified and isolated polypeptide,

- (a) having an amino acid sequence as shown in SEQ ID NO: 6B, or
- (b) having at least 75% homology with the amino acid sequence SEQ ID NO: 6B, or
- (c) being a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has alcohol dehydrogenase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

According to an eleventh aspect the invention provides an isolated DNA sequence encoding

- (a) a polypeptide having an amino acid sequence selected from the group consisting of sequences SEQ ID NO: 7B, 8B, 9B and 10B, or
- (b) a polypeptide having at least
 - i) 55% homology with the amino acid sequence of a 326 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 7B, or
 - ii) 75% homology with the amino acid sequence of a 278 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 8B, or
 - iii)... 65% homology with the amino acid sequence of a 284 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 9B, or
 - iv) 80% homology with the amino acid sequence of a 188 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 10B, or

5 (c) a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has alcohol dehydrogenase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

In particular the invention provides an isolated DNA sequence having a nucleic acid sequence

10 (a) selected from the group consisting of sequences SEQ ID NO: 7A, 8A, 9A and 10A, which encodes a polypeptide having alcohol dehydrogenase activity and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, or

15 (b) complementary to SEQ ID NO: 7A, 8A, 9A or 10A, or

(c) which has at least 55% homology, preferably at least 65% homology, with any of the sequences of (a) or (b), or

20 (d) which is capable of hybridising under stringent conditions to any of the sequences (a) - (c).

Further the invention provides a purified and isolated polypeptide,

25 (a) having an amino acid sequence selected from the group consisting of sequences SEQ ID NO: 7B, 8B, 9B and 10B, or

(b) having at least

30 i) 55% homology with the amino acid sequence of a 326 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 7B

ii) 75% homology with the amino acid sequence of a 278 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 8B, or

35 iii) 65% homology with the amino acid sequence of a 284 aa fragment from the C

terminal end of the coding sequence of
SEQ ID NO: 9B, or

5 iv) 80% homology with the amino acid se-
quence of a 188 aa fragment from the C
terminal end of the coding sequence of
SEQ ID NO: 10B, or

10 (c) being a fragment of polypeptide (a) or (b),
which polypeptide or fragment of polypeptide
has alcohol dehydrogenase activity and is
involved in the biosynthetic pathway for ali-
phatic and/or aromatic ester production in
fruit.

According to a twelfth aspect the invention provi-
des an isolated DNA sequence encoding

15 (a) a polypeptide having an amino acid sequence as
shown in SEQ ID NO: 11B, or

(b) a polypeptide having at least 75% homology with
the amino acid sequence of a 181 aa fragment
from the C terminal end of the coding sequence
20 of SEQ ID NO: 11B, or

(c) a fragment of polypeptide (a) or (b),
which polypeptide or fragment of polypeptide
has alcohol dehydrogenase activity and is
involved in the biosynthetic pathway for ali-
25 phatic and/or aromatic ester production in
fruit.

In particular the invention provides an isolated
DNA sequence having a nucleic acid sequence

30 (a) as shown in SEQ ID NO: 11A which encodes a
polypeptide having alcohol dehydrogenase acti-
vity and being involved in the biosynthetic
pathway for aliphatic and/or aromatic ester
production in fruit, or

(b) complementary to SEQ ID NO: 11A, or

35 (c) which has at least 48 % homology with any of
the sequences of (a) or (b), or

(d) which is capable of hybridising under stringent
conditions to any of the sequences (a) - (c).

Further the invention provides a purified and isolated polypeptide,

- (a) having an amino acid sequence as shown in SEQ ID NO: 11B, or
- 5 (b) having at least 75% homology with the amino acid sequence of a 181 aa fragment from the 3' end of SEQ ID NO: 11B, or
- 10 (c) being a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has alcohol dehydrogenase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

According to a thirteenth aspect the invention 15 provides an isolated DNA sequence encoding

- (a) a polypeptide having an amino acid sequence selected from the group consisting of sequences SEQ ID NO: 12B and 13B, or
- 20 (b) a polypeptide having at least
 - i) 55% homology with the amino acid sequence of a 176 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 12B, or
 - 25 ii) 35% homology with the amino acid sequence of a 284 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 13B, or
- 30 (c) a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has alcohol dehydrogenase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

In particular the invention provides an isolated 35 DNA sequence having a nucleic acid sequence

- (a) selected from the group consisting of sequences SEQ ID NO: 12A and 13A, which encodes a polypeptide having alcohol dehydrogenase activity

and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, or

- 5 (b) complementary to SEQ ID NO: 12A or 13A, or
- (c) which has at least 20% homology, preferably at least 30% homology, with any of the sequences of (a) or (b), or
- (d) which is capable of hybridising under stringent conditions to any of the sequences (a) - (c).

10 Further the invention provides a purified and isolated polypeptide,

- (a) having an amino acid sequence selected from the group consisting of sequences SEQ ID NO: 12B and 13B, or
- 15 (b) having at least
 - i) 55% homology with the amino acid sequence of a 176 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 12B, or
 - ii) 35% homology with the amino acid sequence of a 284 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 13B, or
- 20 (c) being a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has alcohol dehydrogenase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.
- 25

According to a fourteenth aspect the invention further provides a method for regulating aliphatic and/or aromatic ester formation in fruit comprising inserting into the genome of a fruit-producing plant one or more copies of one or more DNA sequences as defined above encoding various enzymes involved in the biosynthetic pathway for aliphatic and/or aromatic ester formation in fruit.

The levels of protein may be increased for example by the incorporation of additional genes. The additional genes maybe designed to give either the same or different

spatial or temporal patterns of expression in the fruit as the target gene.

Further the invention provides a genetically modified strawberry or lemon plant and propagating material 5 derived therefrom which has a genome comprising an expression vector for overexpression or downregulation of an endogenous strawberry or lemon plant gene counterpart of any of the DNA sequences as defined above encoding various enzymes involved in the biosynthetic pathway for aliphatic 10 and/or aromatic ester formation in fruit.

Further the invention provides a method for producing aromatic and/or aliphatic esters in microorganisms, plant cells or plants comprising inserting into the genome of the microorganism or plant one or more copies of DNA 15 sequences of this invention encoding a polypeptide having alcohol acyl transferase activity, and one or more copies of DNA sequences of this invention encoding a polypeptide having alcohol dehydrogenase activity, and feeding aldehydes and acyl-CoA to the microorganism or plant (cell).

20 In a further embodiment the above method further comprises inserting one or more copies of DNA sequences of this invention encoding a polypeptide having pyruvate decarboxylase activity, and feeding alpha-keto acids and acyl-CoA to the microorganism or plant (cell).

25 In a still further embodiment the above method further comprises inserting one or more copies of DNA sequences of this invention encoding a polypeptide having aminotransferase activity, and feeding amino acids and acyl-CoA to the microorganism or plant (cell).

30 In a still further embodiment the above method further comprises inserting one or more copies of DNA sequences of this invention encoding a polypeptide having thiolase activity, and feeding amino acids and fatty acids to the microorganism or plant (cell).

35 Strawberry and citrus are among the most popular fruits for natural flavour ingredients because of their flavour, fragrance, aroma and scent. Examples of commercially important aliphatic esters are ethyl acetate (used in

artificial fruit and brandy flavours), ethyl butyrate (the primary constituent of apple aromas), isoamyl acetate (the main component of banana aroma) and ethyl butyrate (has a fruity odour, reminiscent of pineapple). Flavour usage of 5 these esters is among the highest of all natural flavour ingredients. The invention further contemplates the use of the polynucleotide sequences according to the present invention, for the industrial production of 'fruit' flavours which are natural to match the odour fidelity of the natural 10 fruit. As the chemical composition of flavour is quite complex, both the type and ratio of compounds present being the key determinant of flavour quality, this present invention further contemplates the production of novel flavours by the use of the polynucleotide sequences according to the 15 present invention, alone or in combination, to provide novel avenues for natural flavour production in the future.

The invention further contemplates the generation of strawberry and lemon plants which amongst other phenotypic modifications may have one of the following characteristics:

- (a) reduced/enhanced flavour from the reduced/enhanced production of volatile esters,
- (b) increased disease resistance due to the enhanced production of volatile esters,
- 25 (c) modified plant-insect interactions, to include increased insect resistance.

In a further aspect, the gene sequences of the present invention are used for the manipulation of flavour in other 'fruits' and or/ industrial processes. The genes/ 30 peptides of the present invention, in particular SEQID 1A (strawberry SLE27) and SEQ ID 1B (lemon CLF26) may be used in the following industrial applications; with particular reference to the manipulation of fruit derived products, however, not exclusively limited to these:

- 35 a) in the processed food industry as food additives to enhance the flavour of syrups, ice-creams, frozen desserts, yoghurts, confectionery, and like products,

- b) flavouring agents for oral medications and vitamins,
- c) providing additional flavour/aroma in beverages, including alcoholic beverages,
- 5 d) enhancing or reducing fruit flavour/aroma/france/scent,
- e) enhancing the flavour/aroma of 'natural products (eg flower scent however, including but not exclusively limited to flower scent),
- 10 f) enhancing the flavour/aroma of 'synthetic or artificial products',
- g) as an alternative to the industrial synthesis of nature identical flavour/aroma substances,
- h) for the production of novel combinations of artificial flavour substances,
- 15 i) as antibacterial or as anti-fungal agents,
- j) fragrance /perfumes in the cosmetics, creams, sun-protectant products, hair conditioners, lengthening agent and fixative in perfumes, suspension aid for aluminium salts in anti-perspirants pharmaceuticals, cleaning products, personal care products and animal care products,
- 20 k) disinfectant additives,
- l) as degreasing solvents for electronics (etc.),
- 25 m) insect pheromones,
- n) dye carrier, solvents, insect repellent, miticide, scabicide, plasticiser, deodorants.

An example as way of illustration of the industrial importance of volatile esters (aromatic and aliphatic) 30 is the ester methyl anthranillate. Methyl anthranillate, a methyl ester of o-aminobenzoic acid, apart from being a very important perfumery, aroma, scent ester (it imparts a peculiar aroma known as "foxy" to grapes of the North american species *Vitis Labrusca*), the same compound is known 35 to serve as a pheromone in various insects. Results of investigations aimed at testing the attractiveness of methyl anthranilate for the soybean beetle *Anomala rufocurea*, Motschulsky suggested the potential use of methyl anthrani-

late as a lure for mass trapping, as well as for monitoring (Imai et al., Applied Entomology and Zoology, 32: 1, 45 - 48, 1997). In addition methyl anthrenilate acts as a bird repellent. According to the invention we propose the use of 5 the SEQ1D: 2A for the production of methyl anthranilate, for a number of applied applications.

The present invention also relates to antibodies which specifically bind to the polypeptides as claimed in the invention, compositions comprising substantially purified polypeptides, fragments thereof, agonists or alternatively antagonists, and methods of producing polypeptides. In 10 bacterial/prokaryote systems a number of expression vectors may be selected depending on the used of the polypeptide(s) according to the invention. For example, when large quantities of the polypeptide are needed for the induction of the 15 antibody, vectors which drive high levels of expression of fusion proteins that are readily purified are desirable. Such vectors include, but are not limited to the multi-functional *E.coli* cloning and expression vectors, such as 20 Bluescript® (Stratagene), in which the gene sequence(s) according to the invention, can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that the hybrid 25 protein is produced; pPIN vectors (Van Heekle and Schuster (1989) *J Biol. chem.* 264: 503-5509); and the like. pGEX vectors (Promega, Madison WI) may also be used to express 30 foreign polypeptides as fusion proteins with glutathione-S-transferase (GST). In general such fusion proteins are soluble and can be easily purified from lysed cells by absorption to glutathione-agarose beads followed by elution 35 in the presence of free glutathione. Proteins made in such systems are designed to include heparin or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In addition to recombinant production the amino acid sequences of the polypeptides, or any parts thereof, may be altered by direct synthesis or by genetic means known by those skilled in the art, and/or combined using chemical

methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide. Fragments of the polypeptides according to the invention may be produced by direct peptide synthesis using solid phase techniques (c.f. 5 Stewart et al. (1969) *Solid Phase Peptide Synthesis*, WH Freeman Co, San Fancisco, Merrifield J (1963) *J Am Chem Soc* 85:2149-2154). *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 10 431A Peptide Synthesizers (Perkin Elmer, Forster City CA) in accordance with the instructions provided by the manufacturer. Various fragments of each of the polypeptides may be chemically synthesised separately and combined using chemical methods to produce full length molecules.

15 The invention further contemplates a diagnostic assay using antibodies raised to each of the polypeptides of the present invention, with or without modification. For example, although not exclusively limited to, alcohol acyl transferase antibodies raised to the polypeptides as outlined in the 6-8th embodiments are useful for the detection of flavour forming esters in various fruits. Diagnostic assays for alcohol acyl transferase include methods utilising the antibody and a label to detect the alcohol acyl transferase protein in various fruit extracts. Frequently the polypeptides and antibodies are labelled by joining them, either covalently or non covalently, with a reporter molecule. A variety of reporter molecules are known and several of them have been previously described (cf. reporter genes).

20 A variety of protocols for measuring the polypeptide, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Polynucleotide sequence(s) of the invention, SEQ ID 1A strawberry (SLE27), SEQ ID 2A, lemon alcohol acyl transferase proteins (CLF26), or any part thereof, may be used for 25 diagnostic purposes, to detect and quantify gene expression in various tissues (eg leaves, stems and in particular fruits (climacteric and non climacteric)), hence volatile ester (aliphatic and aromatic) formation, hence flavour at

various stages of plant maturation. The diagnostic assay is useful to distinguish between absence, presence and excess expression of the alcohol acyl transferase proteins and to monitor expression levels during development.

5 The following examples are offered by way of illustration and not by limitation.

Example 1: Method for the detection of volatiles

The composition of the headspace of the strawberries was analysed using Solid Phase Micro-extraction for trapping and 10 concentrating the emitted volatiles. The fibres used were 100 μm Polydimethyl siloxane (PDMS) supplied by Supelco. Analysis was performed on a Fisons GC 8000 system, coupled to the Fisons MD800 mass spectrometer. The gas chromatograph was temperature programmed, with a plateau at 80 °C for 2 15 min., followed by a ramp of 8°C/min to 250 °C for 5 min. The injector port was operated at 250°C for thermal desorption during 60 sec. of the SPME fibers, using the deactivated standard 0.7 ml split/splitless injection port liner with zero split. The column used was a HP-5 with a length of 20 50 m and internal diameter of 0.32 mm and a film thickness of 1.03 μm , and operated with helium at a pressure of 38kPa. Eluted components were identified by their mass spectrum, 25 using the Fisons Masslab software and the NIST library. The identity of the components was verified where possible by using known substances as reference (most of them obtained from Aldrich).

Headspace analyses were performed both on the plant and in the laboratory using detached fruits. The fruits were put onto new clean aluminium foil and covered with a glass 30 funnel closed with aluminium foil. The SPME fiber was inserted in the funnel through the foil closing, and the fiber was exposed to the air in the funnel during 30 min. Figure 2 shows a typical chromatogram obtained after headspace measurement of red ripe fruit of strawberry grown in 35 the greenhouse. The importance of esters to the overall

volatile emission from the ripe strawberry fruit can be clearly observed.

Example 2: Construction of a strawberry red fruit stage and lemon young fruit peel cDNA library, mass excision and 5 random sequencing

2.1 Messenger RNA isolation and cDNA libraries construction
Total RNA was isolated from strawberry fruit red stage of development using the method described by Manning K. [Analytical Biochemistry 195, 45-50 (1991)]. The cultivar used was 10 *Fragaria X ananassa* Duch. cv. *Elsanta*. Plant material for the isolation of RNA from lemon (*citrus Limon* cv. *Meyer*) was the green peel of a small fruit (1.2 x 2 cm) of the cultivar. Isolation of RNA from lemon was done using the same method as described for strawberry. The cDNA libraries for 15 both strawberry and lemon where produced as a custom service by (Stratagene) in the lambda zap vector. Messenger RNA was isolated from total RNA using the polyA+ isolation kit (Pharmacia).

2.2 Mass excision and random sequencing

20 The ExAssist™/SOLR™ system (Stratagene) was used for mass excision of the pBluescript SK(-) phagemid. The excision was done according to the manufacturer's instructions using 20 x 10³ pfu from the non amplified library for each excision. High quality plasmid DNA from randomly picked colonies was 25 extracted using the QIAGEN BioROBOT 9600. Colonies were grown overnight in 3ml Luria Broth medium (10g/l tryptone, 5 g/liter yeast extract, 5 g/liter NaCl) supplemented with 100 mg/litre Ampicillin, centrifuged at 3000 RPM for 10 min. and the pellet was used directly for plasmid DNA isolation by 30 the robot. Each DNA isolation round consisted of 96 cultures.

Insert size was estimated by agarose gel electrophoresis after restriction enzyme digestion of the pBlue-Script (SK-) vector with EcoRI and XhoI. Inserts with length

above 500 bp were used for sequencing. Plasmid DNA from the selected samples were used for polymerase chain reaction (PCR) sequencing reactions using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit and the MJ Research PTC-200 DNA Engine™ thermal cycler. The T3 and T7 universal primers were used for sequencing from the 5' and 3' ends respectively. PCR program was according to the Dye Terminator manufacturer's protocol (ABI PRISM). The ABI 373, 370A and 310 sequencers (Applied Bio-systems) were used. Sequences were edited manually to remove vector and non reliable sequences and submitted to the Blast homology search program using BlastX and BlastN (Altschul et al. *J. Mol. Biol.* 215, 403 - 410, 1990) provided by the National Centre for Biotechnological Information on the world wide web. The strawberry and lemon sequencing data received from the Blast search was transferred using a self designed macro program to a database made using the software Access (Microsoft). Sequences with a BlastX score above 80 were considered as having a significant homology (Pearson, *Curr. Opinion. Struct. Biol.*, 1, 321 - 326, 1991) and they were used for data interpretation in the Access and Excel programmes (Microsoft). The cDNA clones: SLE27 (SEQ ID NO: 1A), CLF26 (SEQ ID NO: 2A), SLF96 (SEQ ID NO: 3A), SLG150 (SEQ ID NO: 4A), SLH51 (SEQ ID NO: 5A), SLB39 (SEQ ID NO: 6A), SLF193 (SEQ ID NO: 7A), SLF122 (SEQ ID NO: 8A), SLD194 (SEQ ID NO: 9A), SLF17 (SEQ ID NO: 10A), SLF138 (SEQ ID NO: 11A), SLG16 (SEQ ID NO: 12A), SLG144 (SEQ ID NO: 13A) were selected for further analysis.

Example 3: Characterisation of selected cDNAs

30 3.1 Sequencing the entire length cDNAs

Plasmid DNA from the selected cDNAs was used for PCR sequencing reactions using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit and the MJ Research PTC-200 DNA Engine™ thermal cycler. Each individual selected cDNA 35 was sequenced completely on both strands. DNA and protein

alignment 'contigs' were performed using the GeneWorks software (IntelliGenetics, Oxford).

3.2. RNA isolation from different tissues and organs of
5 strawberry and lemon.

RNA isolation from both strawberry and lemon tissues was done as described for the preparation of total RNA for the construction of the cDNA libraries [Manning K. *Analytical Biochemistry* 195, 45-50 (1991)]. For both species green 10 house grown plant material was used. From strawberry the different tissues/organs used for RNA isolations were; leaf, root, petiole, flower, green fruit, white fruit, turning fruit, red fruit, red fruit without seeds, seeds and overripe fruit. For lemon the different tissues/organs used were; 15 root, leaf, flower bud, albedo, green fruit peel (1.2 x 2 cm), green fruit peel (2 x 3 cm), green fruit peel (3.5 x 6 cm), green fruit peel (6 x 8 cm), yellow fruit peel (2 weeks fruit detached).

3.3 Expression analysis using Northern blots

20 Ten μ g of each RNA sample was denatured by glyoxal (1.5M) prior to electrophoresis on a 1.4% agarose gel in 15mM sodium phosphate buffer pH = 6.5 (without Ethidium bromide). Equal loading of RNA was verified by visualising equal amount of RNA from each sample on a 1% TAE buffer [1x : 25 0.04M Tris-acetate / 0.001M ethylenediaminetetra-acetate (EDTA)] agarose gel prior to loading. Blotting was done using Hybond N⁺ membrane (Amersham) in 25 mM Sodium phosphate buffer pH = 6.5 overnight.

After fixation (2h 800c) blots were hybridised as 30 described by Angenent et al. 1992 (*Plant Cell* 4, 983-993). The hybridisation probes were made by random labelling oligonucleotide priming (Feinberg and Vogelstein, *Anal. Biochem.* 137, 266-267, 1984) of the entire cDNAs. The hybridised membranes were autoradiographed at -70°C with 35 intensifying screens. In Figure 3 the expression pattern of

strawberry alcohol acyl transferase (SLE27, SEQ ID NO: 1A) is demonstrated by a Northern blot loaded with total RNA extracted from 10 different plant tissues and/or organs from strawberry. The expression pattern of strawberry alcohol acyl transferase is in perfect match with the accumulation of volatile esters in the strawberry plant. The gene SLE27, (SEQ ID NO: 1A) is expressed at its lowest level in the white fruit, gradually increasing as fruit develops reaching its highest levels in the turning stage and the red stage when the volatile ester compounds can be detected at their maximum. In other strawberry plant tissues/organs which do not produce those volatile fruity esters the expression of the gene can not be detected even when the film is exposed to a longer period after hybridisation.

The expression of the lemon alcohol acyl transferase (CLF26 SEQ ID NO:2A) seems to be correlated with the accumulation of volatile monoterpene compounds in the lemon tree tissues/organs (Figure 4). These compounds can be converted by this enzyme to their ester derivatives as a result of alcohol acyl transferase enzyme activity. The gene is more highly expressed in the flower bud. Analysis by Gas chromatograph (GCMS) shows high concentrations of terpene compounds produced in the flower bud such as limonene. Expression analysis of monoterpene cyclases (genes directly responsible for the formation of monoterpenes) isolated from lemon thus shows a similar pattern of expression to the lemon alcohol acyl transferase (CLF26, SEQ ID NO:2A). Expression of the gene was also strongly detected in the early stages of fruit development and in the flower bud whilst no expression could be detected in the root and albedo tissues. This data provides evidence for a link between the presence of terpene compounds mainly monoterpenes and the lemon alcohol acyl transferase (CLF26, SEQ ID NO:2A).

The expression of other three genes part of the metabolic pathway described in this invention for the formation of volatile ester compounds, in particular aliphatic esters in fruits is shown in Figure 5. Thiolase (SLG150,

SEQ ID NO: 4A) alcohol dehydrogenase (SLB39, SEQ ID NO: 6A) and pyruvate decarboxylase (SLH51, SEQ ID NO: 5A) gene expression was monitored at different organs or tissues of the strawberry plant. The data shown provides evidence that 5 thiolase (SLG150, SEQ ID NO: 4A), alcohol dehydrogenase (SLB39, SEQ ID NO: 6A) and pyruvate decarboxylase (SLH51, SEQ ID NO: 5A) are involved in ripening processes such as the production of strawberry flavour and aroma compounds. Clones SLF193 (SEQ ID NO: 7A), SLF122 (SEQ ID NO: 8A), 10 SLD194 (SEQ ID NO: 9A), SLF17 (SEQ ID NO: 10A), SLF138 (SEQ ID NO: 11A), SLG16 (SEQ ID NO: 12A), SLG144 (SEQ ID NO: 13A) and SLF96 (SEQ ID NO: 3A) were also tested for the expression and they were all appearing to have upregulated expression in the ripening fruit (data not shown).

15 3.4 Co-ordinate expression of genes in the biosynthetic pathway leading to aromatic and aliphatic ester formation

The co-ordinate expression pattern of genes of the biosynthetic pathway leading to aromatic and aliphatic ester formation was simultaneously monitored using arrays of 20 cDNAs [Ermolaeva et al., 1998, *Nature Genetics*, Vol, (20), 19-23]. The method allows one to simultaneous quantitatively and statistically analyse the expression profiles of genes pertaining to a particular metabolic pathway, such as pathway leading to aliphatic and aromatic ester formation, 25 flavour, fragrance, aroma and scent. The experimental and hybridisation conditions followed were according to Schena et al., (PNAS, 93: 10614 - 10619). The expression levels derived from four images were statistically analysed with an analysis of variance modelling the factors dye, origin, and 30 spot levels on a logarithmic scale giving the significant ratios of expression between the spots of origin (eg. green versus red). As way of illustration the expression profiles of SEQ ID 1A, SEQ ID 3A, SEQ ID 5A, SEQ ID 11A, SEQ ID 12A, analysed in the green versus red and turning versus red 35 stages of fruit development are outlined in Figure 10. The

co-ordinated expression pattern of the genes during strawberry fruit development is in perfect match with the accumulation of volatile ester compounds aromatic and aliphatic during strawberry fruit development. In addition alcohol 5 acyl transferase enzyme activity was detected by Perez et al., (J. Agric. Food Chem. 1996, 44, 3286-3290) to be elevated dramatically between the white and the turning stages of strawberry fruit development. This correlates well 10 with a dramatic increase in expression of the genes of the biosynthetic pathway as shown in Figure 10. A supporting detail is the fact that volatile ester compounds can be detected in their maximum when the fruit is completely red and this is probably a result of gene activation at earlier stages between the white and turning stage of development.

15 3.5 DNA isolation from strawberry leaf tissue.

Genomic DNA was isolated from green house grown strawberry plants (cv. *Elsanta*). Young folded leaves kept 2 days in water in the dark were used for the isolation. 1 gram of leaf material was ground in liquid nitrogen and immediately 20 added to 7 ml DNA extraction buffer (100 mM Tris-Cl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 0.2% β - mercaptoethanol). After incubation for 30 min. at 600C one volume of chloroform:isoamyl alcohol (24:1) was added to the tubes. The samples were then centrifuged for 10 min. at 4000 rpm and the aqueous 25 phases were transferred to 4.5 ml isopropanol and incubated for 30 min. at -20°C. Nucleic acids were spun down at 4000 rpm for 12 min. washed with 70% ethanol and air dried. Pellets were dissolved in 3 ml TE and 0.25 volumes of NaCl (5M) and 0.1 volume absolute ethanol were added. Samples 30 were centrifuged for 10 min at 4000 rpm and supernatants were transferred to 4.5 ml isopropanol for precipitation. Pellets were washed with 70% ethanol dried and dissolved in 0.5 ml TE buffer (10mM Tris-Cl, 1mM EDTA pH = 8.0). RNA was selectively precipitated by the addition of 0.6 volumes of 35 8M lithium chloride. The RNA was pelleted by centrifugation 10 min. 4000 rpm and the DNA was precipitated again with the

addition of 0.6 volume isopropanol. Pellets were washed with 70 % ethanol, dried and dissolved in 0.5 ml sterile water. The amount of DNA was determined by comparing to known amounts of phage lambda DNA.

5 3.6 Southern blot analysis for strawberry alcohol acyl transferase (SLE27, SEQ ID NO:1A)

Aliquots of 2 μ g strawberry genomic DNA were digested for 16h with the restriction enzymes: HindIII, XbaI, XhoI and EcoRI and separated on a 0.7% TAE (0.04M Tris-acetate pH = 10 8.0 and 1mM EDTA pH = 8.0) agarose gel. The DNA was then denatured in 0.4M NaOH for 30 min. and then transferred to a Hybond N+ membrane in 0.4M NaOH. Blot hybridisation and probe preparation was done as described previously for Northern blots. After hybridisation the blot was washed 15 first under low stringency conditions (52°C with 2 times half an hour 2xSSC/0.1% SDS) and after observation of the results by autoradiography of the film they were washed further under stringent conditions (65°C with 2 times half an hour 0.1xSSC/0.1% SDS) and autoradiographed again.

20 In order to investigate whether the different esters formed in the fruit are a result of an activity of one single enzyme or a multigene family, a Southern blot was performed using strawberry genomic DNA and the full length SLE27 cDNA (SEQ ID NO:1A) which served as a probe (Figure 25 6). Since the cultivar used for the investigation is an octaploid the interpretation of the results is more difficult than that for a diploid plant. However, the results obtained suggest the possible existence of another homolog of strawberry alcohol acyl transferase. This additional copy 30 might arise from one of the parental lines with a similar function to the one isolated, or may represent another homolog with different function or substrate specificity to SLE27 (SEQ ID NO: 1A).

Example 4

Strawberry alcohol acyl transferase (SLE27, SEQ ID NO:1B)
protein analysis

4.1 Cloning to the expression vector pRSET B

5 The *E.coli* expression vector pRSETB (Invitrogen), was used
for expression studies of strawberry alcohol acyl transfe-
rase (SLE27, SEQ ID NO: 1A). This pRSETB vector contains the
T7 promoter which can be induced by isopropyl-beta-D-
thiogalactopyranoside (IPTG) and therefore by inserting the
desired gene downstream of this promoter, the gene can be
10 expressed in *E. coli*. In addition, DNA inserts were posi-
tioned downstream and in frame with a sequence that encodes an
N-terminal fusion peptide. This sequence includes (in 5' to
3' order from the N-terminal to C-terminal), an ATG transla-
tion initiation codon, a series of six Histidine residues
15 that function as a metal binding domain in the translated
protein, the Anti-Xpress epitope, and the enterokinase
cleavage recognition sequence.

20 The original pRSETB was primarily used for the
insertion of the gene encoding the Green Fluorescent Protein
(GFP). The GFP gene was fused to the pRSETB vector using the
BamHI and HindIII restriction sites located at the multiple
cloning site (MCS) as can be seen in Figure 7. This con-
struct for the expression of GFP served as control for the
experiments together with the empty pRSETB vector.

25 Cloning the GFP gene to the pRSETB vector inserted
an additional SalI restriction site at the 3' of the GFP
gene and together with the BamHI site located at the 5' of
the GFP gene served as sites for the cloning of the straw-
berry alcohol acyl transferase gene (SLE27, SEQ ID NO:1A).
30 The BamHI and SalI sites were introduced to the 5' and 3'
respectively of the strawberry alcohol acyl transferase
coding sequence by the use of PCR. The 452 amino acid open
reading frame of the SLE27 (SEQ ID NO:1A) clone was ampli-
fied with the pfu DNA polymerase (Stratagene) and primers
35 AAP165 (5'-CGGATCCGGAGAAAATTGAGGTCAG) and AAP166 (5'-CGTCGA-
CCATTGCACGAGCCACATAATC) according to the manufacturers

instructions. The PCR product was cloned into PCR-script vector (Stratagene), cut out with BamHI and SalI and further inserted (as a translation fusion) into the corresponding restriction sites in the pRSETB vector.

5 4.2 Bacterial expression and partial purification using the His tag columns.

The pRSET B vector harbouring the strawberry alcohol acyl transferase (SLE27, SEQ ID NO: 1A) was used to transform *E. coli* strain BL21 Gold DE3 (Stratagene) as described by the manufacturer. For bacterial expression typically 1 ml of overnight liquid culture grown at 37°C in Luria Broth (LB) medium (10g/l tryptone, 5 g/liter yeast extract, 5 g/liter NaCl) supplemented with 100 mg/liter Ampicillin was diluted 10 times in the same medium and grown until the OD₆₀₀ reached 0.4 (usually at 37°C except when different temperatures for growth during induction were examined). At this stage Isopropylthio-beta-D-galactoside (IPTG) was added to a final concentration of 1mM in order to induce expression . After 15 one and a half hour of induction the cells were harvested by 20 centrifugation at 4000 x g for 15 min. Pellet and a sample from the supernatant were kept for SDS gel analysis. The cells were further processed as described by the Ni-NTA Spin Columns manufacturers (QIAGEN) for protein purification under native conditions.

25 4.3. Recombinant protein detection using Western blot

The supernatant (S), pellet (P) and elute from the Ni-NTA columns (E) samples were diluted with 2x SDS-sample buffer [(standard Laemmli, pH 6.8 Tris /glycine/SDS/glycerol + 50mM Dithiothreitol(DTT)]. The samples were heated for 5-10 min 30 at 95°C, followed by a short spin of 30 seconds, full speed. From the samples 10μl was loaded on 10% SDS-gel (mini-gel, 1mm, 7x9cm). The gels were run with standard buffer (25mM Tris 192mM, glycine 0.1% SDS, pH 8.3) at 100-200V, 50-30mA, ca 3hr with 16°C water cooling. After complete run, gels

were used for Coomassie Brilliant Blue (CBB) stain or Western blot. For CBB staining, the gel was fixed during 1hour in 40% Methanol, 10% Acetic Acid in water. Then incubated overnight with CBB staining solution (0.05% CBB-5 250 in 40% Methanol, 10% Acetic acid in water) and destained by repeated washing in 25% Methanol, 10% Acetic acid in water. Western blot was onto Polyvinyl di fluoride (PVDF), (Immobilon P, Amersham) pre-wetted with Methanol. Transfer buffer was Towbin buffer plus 0.1% SDS (25mM Tris, 192mM 10 Glycine, 20% 0.1% SDS, pH 8.3). Transfer was submarine (Novex blot unit, plate electrodes, 3cm distance) at 200mA overnight plus 2hr. at 400mA, cooled in ice-water with cooling block. Transfer was checked by using pre-stained markers as a reference.

15 After transfer the blot was washed in Tris buffer saline TBS (20mM Tris-HCl pH7.5 / 150mM NaCl) - Tween20 (0.02%), blocked with 2% fat free milk in TBS-Tween20, then washed with TBS-Tween 20. Then first Antibody was anti-Xpress (Invitrogen) 1/1000 in 2% BSA TBS-Tween for 3hr. 20 incubate at room temprature, then 4x washed with TBS-Tween 20. Second Antibody is GAR-AP (Boehringer) 1/3000 in 2% BSA TBS-Tw (TBS + 0.05% Tween - 20) for 1-2hr. incubation at room temprature, then 4x washed with TBS-Tween 20. Final wash was in water, then in Alkaline phosphatase-buffer 25 (100mM Tris pH9.2, 1mM MgCl₂). The staining reaction was with nitroblue tetrazolium/5-bromo-4-chloro-3-indolphosphate/alkaline phosphatase-buffer (45 μ l nitroblue tetrazolium 30mg/ml in 70% dimethylformamide, 33 μ l 5-bromo-4-chloro-3-indol phosphate 15mg/ml in dimethylformamide in 10ml of 30 alkaline phosphatase buffer). The staining reaction was stopped by rinsing with water. Western blotting was used in order to determine the amount of the recombinant SLE27 (SEQ ID NO: 1B) protein in the elute after the Ni-NTA column purification compared to the pellet and supernatant before 35 loading on the column. Figure 9 shows a western analysis of the pellet supernatant and elute after partial purification on the Ni-NTA column. 4 samples were loaded from each pellet, supernatant and elute originating from 4 different

growth and induction of the *E. coli* cells. A commercial antibody recognising an epitope peptide fused to the N-terminus of the SLE27 recombinant protein showed relatively high expression of SLE27 (SEQ ID NO:1B) in the elute from the Ni-NTA column. The estimated size of the partial purified recombinant SLE27 (SEQ ID NO:1B) protein seems to be approximately 60kDa (minus the fusion peptide at the N-terminus. As a control for the experiment served the Green Fluorescent Protein (GFP) partially purified in the same method as done for SLE27 (SEQ ID NO:1B). No band was detectable at the level of the SLE27 (SEQ ID NO:1B) recombinant protein in the GFP lane.

4.4 Method for establishing alcohol acyl transferase activity of the strawberry SLE27 (SEQ ID NO:1B)-encoded protein.

The alcohol acyl transferase activity of the strawberry cDNA SLE27 (SEQ ID NO:1A) encoded protein was established by analysing the volatile esters produced from acyl-CoA and different alcohols. In a 1.8 ml glass vial with a small magnetic stirring bar, 325 μ l of buffer (50 mM Tris/HCl pH 8.0, containing 1 mM dithiothreitol), 25 μ l of protein extract and 25 μ l of acyl-CoA (4 mM in buffer) were subsequently pipetted. The enzyme reaction was started by the addition of 25 μ l of alcohol solution (160 mM in buffer). The vial was quickly closed with a snap cap and placed in a waterbath (at 35°C) with a stirring device. After 15 min enzyme reaction with continuous stirring, the vial was opened, 0.29 g of solid CaCl_2 was added (final concentration 5M) and the vial was quickly closed again with an open top crimp cap. The high CaCl_2 concentration was used to stop further enzymatic reactions and to drive the volatiles into the headspace. After addition of CaCl_2 , the mixture was incubated again at 35°C while stirring. The volatile components released into the headspace were trapped using solid

phase micro-extraction (SPME; Supelco Inc., Bellefonte, PA, USA), exposing the fused silica fiber coated with 100 μ m polydimethylsiloxane (PDMS; Supelco Inc.) to the headspace for 15 min. The presence of esters in the alcohol stock 5 solutions and the spontaneous formation of esters were checked by adding 25 μ l of buffer instead of protein extract into the reaction vial.

The volatiles trapped by SPME were separated and identified by gas chromatography (GC 8000 Series; Interscience B.V., Breda, The Netherlands) coupled to mass spectrometry (MD-800, Fisons Instruments, Interscience) as described by Verhoeven et al. (1997) [Chromatographia Vol. 46, No 1,2]. Compounds were thermally desorbed (2500C) from the SPME-fiber for 1 min. Separation of compounds was on a 15 capillary HP-5 column (50 m x 0.32 mm, film thickness 1.05 μ m; Hewlett Packard) with He (37 kPa) as a carrier gas. The oven temperature was programmed at 2 min at 80°C, then an increase of 8°C min^{-1} to 250°C, and finally 5 min at 250°C. Mass spectra of eluting compounds were generated at 70eV 20 each second. The MD 800 Masslab software (Interscience) was used to record mass spectra and to calculate peak areas. Volatile compounds were identified by screening the NIST library for comparable mass spectra and by comparison with authentic reference compounds.

25 **4.5. Substrates used by the strawberry alcohol acyl transferase-encoded enzyme (SLE 27: SEQ ID NO: 1B)**

The histidine-released protein elute from *E. coli* expressing the strawberry alcohol acyl transferase gene (SLE27, SEQ ID NO:1A) was incubated with both acetyl-CoA and 1-butanol. The 30 chromatograms obtained by SPME of volatiles released into the headspace indicated a clear peak of acetic acid 1- butyl ester (Figure 8A). This ester was not detected when the enzyme was inactivated by freezing and subsequent thawing of the elute (Fig 8B). Moreover, when the SLE27 (SEQ ID NO:1B)- 35 encoded protein elute in the assay was replaced by an equal amount of histidine-released elute from lysate of *E. coli*

expressing only the histidine-tag (i.e. the same vector without the SLE27 (SEQ ID NO:1A gene), the ester could not be detected either (Fig 8C). To investigate which substrates can be used by the SLE27 (SEQ ID NO:1B)-encoded protein to produce volatile esters with acetyl-CoA, the alcohol species in the assay was varied. Table 1 shows all substrates tested and the species that can be used by the SLE27-encoded enzyme (SEQ ID NO:1B). Clearly, this enzyme can use both aliphatic and aromatic alcohols.

10 Table 1. An illustration of some of the different ester products formed by the SEQ ID: 1A, the strawberry alcohol acyl transferase (SLE27) as detected by GCMS analysis, after heterologous expression in *E.coli*.

Table 1

15	Acyl CoAs	Alcohol	Ester formed
	acetyl CoA	1 - propanol	1 - propyl acetate
	acetyl CoA	1 - butanol	1 - butyl acetate
	acetyl CoA	2 - butanol	2 - butyl acetate
	acetyl CoA	furfuryl alcohol	furfuryl alcohol acetate
20	acetyl CoA	iso propanol	isopropylacetate
	acetyl CoA	1 - hexanol	hexyl acetate
	acetyl CoA	1 - octanol	n-octyl acetate
	acetyl CoA	phenylethyl al- cohol	acetic acid phenylethyl ester
	acetyl CoA	benzyl alcohol	acetic acid phenylmethyl ester
25	butyl CoA	1 - propanol	butanoic acid propyl es- ter
	hexyl CoA	1 - propanol	hexanoic acid propyl es- ter

Sequence listing

INFORMATION FOR SEQ ID NO: 1A (SLE27)

SEQUENCE CHARACTERISTICS:

LENGTH: 1638

TYPE: cDNA

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL ACYL TRANSFERASE

SEQUENCE DESCRIPTION FOR SEQ ID NO: 1A

ACCTACTTGCCTTAAACATGGAGAAAATTGAGGTCAGTATAAATTCCAAACACCAACATCAACTTCCTCTACACCACTTCAGCCTTACAAGCTTACCTCCTGGACCAGCTCACTCCTCCGGCGTATGTCCCCATCGTGTCTTCTACCCTCATTACTGACCATGACTCAATCTCCTCAAACCCCTAGCTGACTTAAGACAAGCCCTTCGGAGACTCTCACTTGTACTATCCACTCTGGAAAGGGTCAAAAACAACCTATACATCGATGATTGAGAAGAAGGTGTCCCACACCTTGGGCTCGAGTGAATTGTGACATGACTGATTGAGGCTTCGGAAAATCGAGTGCCTTAATGAGTTGTTCCAATAAAACCATTAGTATGGAAGCAATATCTGATGAGCGTTACCCCTGCTGGAGTTCAAGTCACGTTTCGATTCTGGAATAGCAATCGGTGTCTCCGTCTCTCACAAAGCTCATCGATGGAGGAACGGCAGACTGTTCTCAAGTCCTGGGGTGCTGTTTCGAGGGTGTCTGAAATATCATAACATCCTAGTCTCTGAAGCAGCATTGCTTCCACCGAGAGATGACTTGCCTGAAAAGTATGTCGATCAGATGGAAGCGTTATGGTTGCCGGAAAAAAAGTTGCTACAAGGAGATTGTATTGGTGTGAAAGCCATCCATATCTCAATTCAAGATGAAGCGAAGAGCGAGTCCGTGCCAAGCCATCACGAGTTCATGCCGTCACTGGTTCTCTGGAAACATCTAATCGCTGCTTCACGGGCACTAACATCAGGTACTACTTCACCAAGACTTCTATAGCGGCC-

CAGGCAGTGAACCTAACAGAACACGGATGAACATGGAGACAGTGTGGATA-
ATGCCACTGGAAACTTGTCTGGTGGCACAGGCCATACTAGAGCTAAGT-
CATACAACACCAGAGATCAGTGATCTAACAGCTGTGACTTGGTTAACTTG-
CTCAATGGATCTGTCAAACAATGTAACGGTGATTACTTGAGACTTCAG-
GGTAAAGAGGGATATGGAAGAATGTGCGAGTATCTAGATTTCAGAGGAC-
TATGAGTTCTATGGAACCAGCACCGGATATTATTATTCTCGAGCTGGAC-
TAATTTTCACCCACTTGATTTGGATGGGGAGGACATCATGGATTG-
GAGTTGCAGGAAAAATTGAATCTGCAAGTTGCAAGTTCTAACATATTAGTC-
CAACACAATGCGGTTCTGGAATTGAAGCGTGGGTGAATCTAGAAGAAGA-
GAAAATGGCTATGCTAGAACAAAGATCCCCATTTCTAGCGTTAGCATCTC-
CAAAGACCTTAATTAAAGATATTGATTAAGAAAGATTATGTGGCTCGTG-
CAATGTTCGATTTGCAGTGAATAAGGTTAAATTAGTTACGTATGTTATCCGAATGT-
CAATAAAATGCAAGTATGATAGACTTGTCTACGTATGTTATCCGAATGT-
GTTCCATATGCTTGTAAACCAATATAGCTCTTATTGTAACAAATGCTCTAT-
TAAGCTTCTAGCTATAAGTTATTATCTATTAAAAAAACTATGGAAGT-
TTTACCAAAAAAAAAAAAAAA

INFORMATION FOR SEQ ID NO: 1B (SLE27)

SEQUENCE CHARACTERISTICS:

LENGTH: 452

TYPE: Peptide

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL ACYL TRANSFERASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 1B

MEKIEVSINSKHTIKPSTSSTPLQPYKLTLLDQLTPPAYVPIVFFYPITDHDFNL-
PQTLADLRQALSETLTLYYPLSGRVKNLYIDDFEEGVPYLEARVNCDMTDFL-
RLRKIECLNEFVPIKPFMMEAISDERYPLLGVQNVFDGIAIGVSVSHKLIDGG-
TADCFLKSWGAVFRGCRENIHPSLSEAALLFPPRDDLPEKYVDQMEALWFAG-
KKVATRRFVFGVKAISSIQDEAKSESVPKPSRVHAVTGFLWKHLIAASRALTS-
GTTSTRLSIAAQAVNLRTRMNMETVLDNATGNLFWWAQAILELSHTPEIS-
DLKLCDLVNLLNGSVKQCNGDYFETFKGKEGYGRMCEYLDFQRTMSS-
MEPAPDIYLFSWTNFFNPLDFGWRGRTSWIGVAGKIESASCKFIILVPTQCGS-
GIEAWVNLEEEKMAMLEQDPHFLALASPKTLI

INFORMATION FOR SEQ ID NO: 2A (CLF26)

SEQUENCE CHARACTERISTICS:

LENGTH: 1613

TYPE: cDNA

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: CITRUS LIMON ALCOHOL ACYL TRANSFERASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 2A

CTTATTAAAAGTTCATCAACAAATTGTTCTACCACTTACCATTTCTCATAG-
CTCTGCAAGTTCGGATTGACTCTTCTCTTCTCATTCCGGCCGGTGT-
GATAGTTACATTTGGCACAATGAAAATTACGTTAAGGAGTCAACAATT-
TACGCCCTGCTCAAGAAACACCCAAGCATCGCCTACAAATATCCGACCTA-
GACATGATTGTGCCATCCAATTACGTTCCAGTGTGTATTCTATCGGCG-
GTCCAGTGACTGCACCGATTGGTGAAGTTGGTTGCTGAAGAAGGCTCT-
GAGCGAAGTTCTGTGCCGTTACCCGTTGCCGGAAAGGTTGCAGAAG-
GATGAAAATCGCAAAATTGAGATTCTATGTAACGGAGAGGGAGTTGTT-
TCTGGAGGCCGAAACAAGTTGTGGTATTGATGATTGGTACTCTCA-
CAAGGCTCGAAACTCCTGACGCTTCCAAGTGTGGTATAACAAAGGA-
TATATCCTCCCATCCACTCTGATGGCACAGGTAACCTATTCAAATGTG-
GAGGCCTTGTGGAACTAGAGTGAATCATACTGGTAGATGGAGCTT-
CAGCGTACCATATCATCAACTCATGGCGGAGACGACGCGTGGCGTTCC-
TATTAGCACTCAACCATTCTATGATCGGACCATACTGAGTGTGGGGTTC-
CAACTTCTCCCAAATTCCATCACATTGAATATGACCCGCCTCCTCCAT-

GAACGCTCCTCCTACCCAAAATCCTGAAATCATTCTACCGCAATCCTTAAC-
CTATCACTTGATCAAATCCACACCCTCAAAGAGAAATCTAAGACAGATCAT-
GAACCCAACGTCAAGTATAGTAGGATGGCGATCCTAGCAGCACATATCTGG-
CGTAGCATGTGTAAAGCGCGCGGATTATCTGATGATCAAGTTAGCAAGTTA-
CACTTCCTACAGACGGACGACAGAGATTGAATCCACCACTCCCGCCTGGA-
TATTGGAAATGTAATTTCACCACGTCGTTGACGGCTTCATCGGGTGA-
TATCCTAAGTGAACCATTGAATCATACTGTTGAAAGAATTCAAAAAGCAT-
TAAAGCGGATGGACGATGAGTATTGAAATCAGCACTGCTTACCTAAAG-
CAACAGCCTGATTAAATGCTCTACGGAAAGGAGGCCACATTACAAGTGC-
CCTAACCTCAATATCGTCAATTGGCAAATATGCCAATGTATGTCGAATT-
TTGGATGGGCCAGCGATATTGCGAGGATCGTTAACACACATATTATGAAG-
GGATAGCACATATTATCCAAGTCCGAGCAATGATGGACCTTGTCAAGTGCAGTG-
GTTATAAAACTCGGTAGCCGATCACATGCAGCTGTTCAAGAAGTTCTT-
TACGAGATCTTGATTAAGGTATGAAAGACCTAGGTATTTATATTCTA-
GAAATGTCACTTTTTTTTTTGGGGCGCAAATGTTGTCTTAC-
TTGGAATTATATTTAATCCATGTTTATGGAAGGCAGTGGTGTG-
CAAA

INFORMATION FOR SEQ ID NO: 2B (CLF26)

SEQUENCE CHARACTERISTICS:

LENGTH: 434

TYPE: Peptide

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: CITRUS LIMON ALCOHOL ACYL TRANSFERASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 2B

MKIHVKESTIIRPAQETPKHRLQISDLDMIVPSNYVPSVYFYRRSSDCTDF-
FEVGLLKKALSEVLVPFYPVAGRLQKDENRKIEILCNGEGVLFLEAETSCGIDD-
FGDFSQGSKLLTLVPTVGDTKDISSHPLLMAQVTYFKCGGVCVGTRVNHTL-
VDGASAYHIINSWAETTRGVPISTQPFYDRTILSVGVPTSPKFHHIEYDPPPSM-
NAPPTQNPEIISTAILNLSLDQIHTLKEKSKTDHEPNVKYSRMAILAAHIWRSM-
CKARGLSDDQVSKLHFPTDGRQRLNPPLPPGYFGNVIFTSLTASSGDILSEPLN-
HTVERIQKALKRMDDEYLKSALAYLKQQPDLNALRKGGHIYKCPNLNIVN-
LANMPMYVANFGWGQPIFARIVNTYYEGIAHIYPSPSNDGTLVVINSVADHM-
QLFKKKFFYEIFD.

INFORMATION FOR SEQ ID NO: 3A (SLF96)

SEQUENCE CHARACTERISTICS:

LENGTH: 1586

TYPE: cDNA

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY AMINOTRANSFERASE

SEQUENCES DISCRIPTION FOR SEQ ID NO:3A

AAACCGTCGGCGTCTGTAAATGCGTCGCCGCTCCGGAGAAGACAGAGTA-
CAAGACTCAGGTGAATCGCAATGCCAACATGGCCAAGCTTCAAGCCGGT-
TATCTTTTCCAGAGATTGCGAGGAGGAATGCGCACTTGCAGAACGCAC-
CCTGATGCGAAGATAATTCCACTTGGATTGGTGTACTACCGAGCCAAT-
TCCAGAATATATAACCTCTGCAATGGCAAAGAGAGCACTGCCATGTCCAC-
CCTAGAGGGTTACAGTGGTTATGGACCTGAACAAGGTGAAAAGCCACTGA-
GAGTTGCAATTGCTAAAACGTTTATGGCGACCTGGCATAGAGGAAGAT-
GACATATTGTTCTGATGGGGCAAAATGTGACATATCCCGCTTCAGGT-
TCTTTTGGGGCGATAAAACAATAGCAGTGCAAGATCCATCGTATCCGGC-
TTATGTAGACTCAAGTGTATTATGGGCCAGACAGGACAGTATCAGAAATC-
TGTTCAAGTTGGAAACATCGAGTACATGAGGTGTACTCCGATAATG-
GATTTTTCTGATCTGTCCTCTACTAAGCGAACAGATATCATATTTCT-
GTTCACCAAACAATCCTACTGGTTCTGCTGCAACAAGGGAGCAACTGACA-
CAACTTGTAAAGTTGCCAAGGATAATGGTTCAATCATAGTCTATGATTCT-

GCATATGCCATGTATATGTCAGATGATAATCCACGCTCCATCTTGAAATCC-
CTGGAGCTAAAGATGTTGCACTTGAGACATCATCATTAGTAAGTATGCCG-
GATTCACTGGAGTTCGTTGGGGTGGACTGTGGTCCAAAGCAGTTGCAG-
TATTCAAGATGGTTTCAAGTTGCCAAGGATTCAACCGCATTGTTGTACTT-
GCTTCAATGGTGCATCCACTATTATCCAAGCTGGTGGTCTGGCTTGCCTT-
CAACCAAAGGGTGTAAAGGCTATGCACGGTGTGATAAAATTCTACAAA-
GAAAATACTAAGATCATAATGGAGACGTTAACTCTCTGGCTTAACGTG-
TATGGAGGGACAAACGCTCCATATGTGTGGGTCCACTCCCTGGACAAAGC-
TCCTGGGATGTGTTGCTGAGATCCTGAGAAGACTCATGTGGTAACCA-
CACCTGGAAGTGGCTTGGACCTGGTGGTGAAGGTTCATCAGGGTAAGTG-
CCTTGGACACAGGAAAAATATATTAGAACATGTAAAAGATTCAAGCAAT-
TATACAAGTGAGGACTGCGGATCTGAATTGTAGACCAGTTCTACTGCATG-
CTAGTTGAACCTATTGCCTCCCATTCCGTTATGCTAAATATTTAG-
CACGTTCCAATTCCGTATTCAAGTTGTCGGCTTAGTTATGAATTATGGA-
GATTTAGCTATTGTAAAAATGATTGATCAGCCTGTTCATGTGTTA-
CACTTAATTGTTGTAACATTGTGAGGATCAGAACGCTTGATTCTGTTGC-
TAGAATAGTATAATTACCTAAATAAAGTGGTTGATCTTCTTGGCCTG-
CAAAAAAAAAAAAAAAAAAA

INFORMATION FOR SEQ ID NO: 3B (SLF96)

SEQUENCE CHARACTERISTICS:

LENGTH: 397

TYPE: Peptide

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY AMINOTRANSFERASE

SEQUENCES DISCRIPTION FOR SEQ ID NO:3B

MAKLQAGYLFP EIARRRNAHLQKHPDAKIIPLGIGDTTEPIPEYITSAMAKRA-
LAMSTLEGYSGYGP EQGEKPLRVIAIAKTFYGD LGIEEDDIFVSDGAKCDISR-
LQVLFGADKTIAVQDPSYPAYVDSSVIMGQTGQYQKSVQKFGNIEYMRCTPD-
NGFFPDLSSTKRTDIIFFCSPNNPTGSAATREQLTQLVKFAKDNGSIVYDSAYA-
MYMSDDNPRSIFEIPGAKDVALETSSFSKYAGFTGVRLGWTVPKQLQYSDG-
FQVAKDFNRIVCTCFNGASTIIQAGGLACLQPKGVKAMHGVINFYKENTKI-
IMETFNSLGFNVYGGTNAPYVWWVHFPGQSSWDVFAEILEKTHVVTTPGSGFGP-
GGE GFIRVSAFGHRKNILEACKRFKQLYK

INFORMATION FOR SEQ ID NO: 4A (SLG150)

SEQUENCE CHARACTERISTICS:

LENGTH: 1775

TYPE: cDNA

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY THIOLASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 4A

CGCTCCTTGATTCCTTGTTCATTCAAGAGTATGGAGAAAGCGAT-
CAACAGGCAGAAGGTTCTCCTCGACCATCTCCGACCTTCTTCTTC-
CGACGACTCTCTCTCCCGCGTCGGTATGTGCGGCTGGGGATAGCGCTG-
CGTATGCTAGGAATCATGTCTTGGGGACGATGTCGTATCGTTGCAGCTT-
TTCGCACTCCACTCTGCAAGGCTAACGCGTGGCGGCTTCAAGTATACTTATG-
CTGATGATCTCCTCGCACCTGTCCTCAAGGCCGTGGTTGAGAAAACCAATC-
TCAATCCAAGGAAGTCGGGGATATTGTTGTCGGTACCGTCTGGCCCCAG-
GATCTCAGAGAGCTAGCGAATGCAGGATGGCTGCTTCTATGCTGGCTTCC-
CTGAGACTGTGCCGGTTAGAACTGTGAACAGACAATGTCGTCTGGCCTC-
CAAGCAGTTGCTGATGTTGCTGCTGCCATTAGAGCAGGGTTTATGATATT-
GGCATTGGTGCTGGTTGGAATCCATGACTGCAAACCCAATGGCATGG-
GAAGGGGATGTTAACCTAAAGTAAAGATCTTGAACAAAGCCCAGAATTGC-
CTTCTCCTATGGGAGTCACCTCAGAAAATGTTGCTCATCGTTGGTGTTC-
CAAGACAGGAGCAAGATCAGGCTGCAGTTGACTCTCATAGAAAGGCAGCT-
GCTGCTGCTGCTGGTAGATTAAAGATGAAATCATCCCTGTGGCAAC-

CAAGATTGTTGATCCAAAATCTGGTGATGAGAAACCTGTTACAATCTCT-
GTTGATGATGGGATTGAAACACAACATTGGCGGACCTAGCAAAGCT-
GAAGCCTGTGTTAAGAAAGATGGGACCACCACTGCTGGTAATTCTAGT-
CAAGTTAGTGATGGTGCTGGAGCTGTTCTGATGAAGAGAAGTGTGC-
CGACCAAAAAGGATTGCCGATTCTGGTGTATTAGGAATTTGTTGCT-
GTTGGTGTGGATCCTGCCATCATGGGTGTTGCCAGCTGCTGCAATTG-
CAGTTGCAGTTAAGGCAGCTGGTTAGAGCTGATGATATTGACCTTTT-
GAGATAAAATGAGGCTTTGCATCCCAATTGTTATTGCCGTAACAAGCTG-
GGACTTGATCCAGAAAAAATCAATGTTAACGGAGGTGCAATGCCATGGC-
CATCCACTTGGTGCAACAGGTGCCGGTGTGTTGCCACTCTTGCATGA-
GATGAAGCGTCGTGGTAAAGACTGCCGCTATGGAGTGATCTCAATGTG-
CATAGGCACAGGGATGGGTGCAGCCGCTGTTTGAAGAGGGAGACCG-
GACCGATGAACTCTGCAATGCTCGCAAGGTTGAATCACTCAACTTCTTATC-
CAAGGATGTTGGTAGTAGAGAATGGTTAGTGACAGGAGCTATTCAATCA-
ATAATGTTGGTGGAGTCTGAAAATCATAGTAAAGCACTGGAATAACGTTG-
CTAAGTTTCGTTGGTACTACCTGTTATTGGGATGGAATACACATG-
TAGTTGGTTGTTCTCCCAGACCTCCACTTGGCATATTCAATTGTC-
CAACCTAAAAAGTCCATTATAGGACTTCATCTCAATAAACATTGGTTG-
GCCACTAAAGCAGTGCCTAAAATGTAATTGGTAATTGGTACACCT-
GTTGCTACTTCTTCTAAGTTAATCAAGCCCTGCCACCTCATA-
TAAAAAAAAAAAAAAAAAAAAAA

INFORMATION FOR SEQ ID NO: 4B (SLG150)

SEQUENCE CHARACTERISTICS:

LENGTH: 458

TYPE: Peptide

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY THIOLASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 4B

MEKAINRQKVLLDHLRPSSSSSDSSLSASVCAAGDSAAAYARNHVFGDDVVI-
VAAFRTPLCKAKRGGFKYTYADDLLAPVLKAVVEKTNLNPKEVGDIVVGTV-
LAPGSQRASECRMAAFYAGFPETVPVRTVNRQCSSGLQAVADVAAIRAGFY-
DIGIGAGLESMTANPMAWEGDVNPKVKIFEQAQNCLLPMGVTSENVVAHRFGV-
SRQEQQDQAAVDSHRKAAAAAAAGRFKDEIIPVATKIVDPKSGDEKPVTISVD-
DGIRNTTLADLAKLKPVFKKDGTAGNSSQVSDGAGAVLLMKRSVADQKGL-
PILGVFRNFVAVGVDPAIMGVGPAAAIPVAVKAAGLELDDIDLFEINEA-
FASQFVYCRNKLGLDPEKINVNGGAMAIGHPLGATGARCVATLLHEMKRRGK-
DCRYGVISMCI GTGMGAAAVFERGDR TDELCNARKVESLNFLSKDVR

INFORMATION FOR SEQ ID NO: 5A (SLH51)

SEQUENCE CHARACTERISTICS:

LENGTH: 2141

TYPE: cDNA

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY PYRUVATE DECARBOXYLASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 5A

ATTTCACTCAGAGTCTCAATCTTCATCACAAAAATTCCCATTGATCA-
CAAAAAAGTTCAACCTTAAACCTCCATGGACACCAAGATTGGCTC-
CATCGACGTCTGAAAACCGAGAACGACGACGTCGGTTGTTACCAAACAG-
CGCCACCTCCACC GTTCAAAACTCAGTCCCTCACCTCCCTCAGCTCCGC-
CGACGCCACCCCTCGGCCACCTGGCACGCCGCCTCGTTCAAATGGCGT-
CACCGACGTCTCACCGTCCCCGGCGACTTCAACTTGACCC TTGACCAC-
CTCATGCCAGCCGGCCTCACCAACATTGGCTGCTGCAACGAGCT-
CAACGCCGGGTACGCCGCCACGGCTACGCCGGTGCCTGGCGTGGCGATCG-
CGTGCCTGGTGACTTCACTGTTGGACTGAGTGTGCTGAACGCGATCG-
CCGGCGCGTATAGTGAGAATTGCCGGTATTGTATTGTTGGTGGGCC-
CAACTCTAACGATTATGGGACTAACCGGATTCTCACCAACTATTGGGTT-
GCCGGACTTCAGTCAAGAGCTCCGGTCTTCAGACCGTGACTGCTTT-
CAGGCTGTGGTGAATAATCTGGAGGATGCACATGAGATGATTGATACTGCA-
ATTGCACTGCGTTGAAAGAAAGCAAGCCTGTGTATATCAGCATTGGCTG-
CAACTGGCTGGGATTCCCTCATCCTACTTCAGCCGTGAACCTGTTCCATT-

TCATTGTCTCCAAAATTGAGCAATAAGTGGGGATTAGAGGCTGCAGTG-
GAGGCTGCTGCAGAGTTCTGAACAAGGCAGTGAAGCCAGTTATGGTGGG-
CGGGCCCAAACGTGCCTCTGCACATGCTGGTATGCCCTTGTGAACGGC-
TGATGCTTCTGGATTGCTCTGGCTGTGATGCCATCAGCAAAGGGGCAAGT-
GCCAGAGCACCACCCCCATTTCATCGGAACGTACTGGGGTGTGAGCAC-
TGCCTTGTGCTGAGATTGTGGAGTCTGCAGATGCATACTGTTGCTG-
GGCCGATTTCAATGACTACAGCTCAGTTGGTACTCGCTCCTCTCAA-
GAAAGAGAAGGCGATCATTGTGCAGCCAGATCGTGTGACGATAGGAAT-
GGCCCTACATTGGTTGTGTTCTCATGAAGGATTCCCTCTAGGCCTAG-
CAAAGAAGCTGAAGCATAACAAACACTGCTCATGAGAACTACCGCAGGATCT-
TTGTGCCTGATGCCACCCCTCTGAAGGCTGCACCCAAAGAACCTTGAGG-
GTTAATGTTCTGTTCAAACACATTCAAATATGCTGTGAGCTGAAACCGCT-
GTGATTGCTGAGACAGGGACTCATGGTTAACTGTCAGAAGCTGAAATTG-
CCACCCGGCTGCGGGTATGAGTTCAAATGCAATATGGATCAATTGGTTG-
GTCAGTTGGAGCAACTCTGGTATGCTCAGGCTGTACCTGAGAAGCGAGT-
GATTCTTCATTGGTATGGAGCTCCAGGTGACTGCTCAAGATGTGTC-
CACAAATGATTGAAATGGACAGAGAACCAATTATTCCTGATAAACAAATG-
GTGGATACACCATTGAAGTGGAAATCCATGATGGACCATAACATGTGAT-
CAAGAACTGGAACTACACTGGACTGGTTGATGCAATCCACAATGGG-
GAAGGCAAGTGCTGGACAACCAAGGTGCGTTGCGAAGAGGGAGCTGATT-
GAAGCAATAGAGACTGCAAATGGACCCAAGAAGGATAGCTTCTGCTTCATT-
GAGGTGATTGTTACAAGGATGATACCAGCAAAGAGTTGCTTGAGTGGGG-
GTCTAGGGTTCTGCTGCCAACAGCCGCCACCTAACCTCAGTAAACACTC-
TCCTGTGTCATATGAAGGCCTTCATTCACACAGATTAGATCAAGC-

CAAGCTCTTGTGCAAATTTCTTATGTTTCTGTCAACTGGAGAATGGT-
GTCTGTCAAGTTTTTACACTACAGTGATTCTGGTTGTCTGTATATT-
TCCTTCTGAATATTAGTATCTTCTGATTTCAATTGATCAAATTCTGT-
GATCCTAAATGGTTGTGGAAAAAAAAAAAAAAA

INFORMATION FOR SEQ ID NO: 5B (SLH51)

SEQUENCE CHARACTERISTICS:

LENGTH: 605

TYPE: Peptide

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY PYRUVATE DECARBOXYLASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 5B

MDTKIGSIDVCKTENHDVGCLPNSATSTVQNSVPSTSLSSADATLGRHLARRL-
VQIGVTDVFTVPGDFNLTLHDHLIAEPGLTNIGCCNELNAGYAADGYARSR-
GVGACVVTFTVGGLSVLNAIAGAYSENLPVICIVGGPNSNDYGTNRILHHTI-
GLPDFSQELRCFQTVTCFQAVVNNLEDAHEMIDTAISTALKESKPVYISIGCN-
LAGIPHPTFSREPVPFSLSPKLSNKWGLEAAVEAAAFLNKAVKPVMVGGPKL-
RSAHAGDAFVELADASGFALAVMPSAKGQVPEHHPHFIGTYWGAVSTAF-
CAEIVESADAYLFAGPIFNDYSSVGYSLLLKEKAIIVQPDRVVTIGNGPTFGCV-
LMKDFLLGLAKKLKHNNTAHENYRRIFVPDGHPLKAAPKEPLRVNVLFK-
HIQNMLSAETAVIAETGDSWFNCQKLKLPPGCGYEFQMQYGSIGWSVGATL-
GYAQAVPEKRVISFIGDGSFQVTAQDVSTMIRNGQRTIIFLINNGGYTIEVEIH-

DGPYNVIKNWNYTGLVDAIHNGEGKCWTTKVRCEEELIEAIETANGPKKDSF-
CFIEVIVHKDDTSKELLEWGSRVSAANSRPPNPQ

INFORMATION FOR SEQ ID NO: 6A (SLB39)

SEQUENCE CHARACTERISTICS:

LENGTH: 1415

TYPE: cDNA

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 6A

TAATCTAGCTTCTGCACCAAAACTATCAGATAATTAAGAACATCTGCCACAGA-GAAAATGGTGATGTCTATCGAGCAGGAACACCCCAAGAACGGCATCTGGAT-GGGCTGCAAGAGAGATTCATCTGGTGTCTCTCCCTCAGTTCTCCAGAAG-GGAAACCGGAGAGAAAGACGTGACGTTCAAAGTGTACTGTGGGATTT-GCCATTGGACCTTCACATGGTCAAGAACATGAATGGGGCTTCTACCTATC-CTCTGGTTCCAGGGCATGAGATTGTTGGTGAAGTGACGGAAGTAGGAAG-CAATGTACAAAAATTCAAAGTTGGAGACAGAGTCGGTGTGGATGCATTGT-GGGATCTTGGCGATCTTGTGAAAATTGTACCGACCACCTTGAGAACTACTG-CCCCAACAGATACTCACTTACGGTGCCAAGTACTACGACGGAACCAC-CTATGGCGGTTACTCTGACATTATGGTGGCCGATGAACACTTCATAGTACG-CATCCCAGACAACCTTGCCTCTTGATGGTGTGCTGCCGCTCCTATGTGCCGG-GATTACAACCTACAGCCCCCTGAGATATTCGGACTTGACAAGCCGGCAT-GCATGTAGGTGTGGTGGCCTAGGCGGTTAGGCCACGTCGCCGTGAAGT-TTGCCAAGGCTATGGGAGTGAAGGTTACAGTGATTAGTACATCCCCTAA-

GAAAGAGGAGGAAGCTCGTAAACACCTAGGAGCTGACTCGTTTGGTTAG-
CCGTGACCAAGATCAAATGCAGGCTGCCATTGGTACCATGGATGGGAT-
CATTGACACGGTTCTGCACAACATCCTCTCCTGCCTTGATTGGTTGTT-
GAAGTCTCATGGAAAGCTTGTATGGTGGTGCACCAGAGAAGCCTCTT-
GAAC TGCCAGTTTCCTTACTCATGGGAAGAAAGATGGTAGCTGGTAG-
CGGCATTGGGGTATGAAGGAGACACAAGAGATGATAGATTTGCAGC-
CAAGCACAACATTACAGCAGACATCGAAGTCATACCAATCGACTACTG-
TAACACTGCTATGGAGCGTCTAGTCAAAGCAGATGTCAGATAACGTTTGT-
CATCGACATTGGAAACACACTGAAGGCTAGCTCTTAAATTCTGCAATCCA-
GACTGGATCAATGAAGAAACAAGAACAGAACGGAGACTGATTAGTGT-
CATACTCGGTGTTGGTTTCTTGTAGCATTGTTGTCTGCTACATGA-
ATAATGATCACATGAACAACTGCCTCTGTGATGATTGATAATAAAAGA-
ATACATGAACAAATGATACTGCCTCTTGTAAATGTTTACTATATAAT-
CATTCAAATTATTTGCTA-
TATCTCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

INFORMATION FOR SEQ ID NO: 6B (SLB39)

SEQUENCE CHARACTERISTICS:

LENGTH: 333

TYPE: Peptide

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 6B

MVMSIEQEHPKKASGWAARDSSGVLSFPSFSRRETGEKDVTFKVMYCGICHS-
DLHMKNEWGFSTYPLVPGHEIVGEVTEVGSNVQFKVGDRVGVCIVGSCR-
SCENCTDHLENYCPKQILTYGAKYYDGTTYGGYSDIMVADEHFIVRIPDNL-
PLDGAAPLLCAGITTYSPLRYFGLDKPGMHGVVGLGLGHVAVKFAKAM-
GVKVTVISTSPKKEEEARKHLGADSLVSRDQDQMQAIGTMDGIIDTVSAQH-
PLLPLIGLLKSHGKLVMVGAPEKPLELPVFPLLMGRKMVAGSGIGGM-
KETQEMIDFAAKHNTADIEVIPIDYL

INFORMATION FOR SEQ ID NO: 7A (SLF193)

SEQUENCE CHARACTERISTICS:

LENGTH: 1227

TYPE: partial cDNA

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DESCRIPTION FOR SEQ ID NO: 7A

GGAAACAGGAGCAACGGACGTAAGATTCAAAGTGTGTACTGTGGAGTAT-
GCCATTGGACATACACATGGCCAAAATGATTGGGGACTTCTACCTATC-
CTATTGTACCTGGGCATGAACCTGTTGGTAGTAAACAGAAGTAGGATG-
CAAAGTAAAGAAATTCAAAAGTTGGAGACAAGGTCGGTGTGGTTGCATG-
GTCGACTCAGACCAACTTGCAGAAATTGTATCCATCACCTAGAAAATTACT-
GTCCGAATCTGATAACAAACCTACGGTTCTAAATACTACGACGGAACCATGA-
CATACGGAGGTTACTCGAACAAACATGGTACTGATGAGCAGTTCATTGT-
TCGGATCCCGGACAACCTACCTCTTGTGGCGCTGCTCCGCTTATGTGC-
CGGGATTACAACCTACAGCCATGGAGATATTATGGACTTGACAAACCCGG-
TATGCATCTTGGTGTGAATGGCCTAGGCGGTTAGGTACGTCCGCCGT-
TAAATTGCCAGGGCTTGGGCTCAAGGTTACAGTCATTAGTACCTCCCC-
TAATTAAAGAAGGAGGCAGCTATGGAACATCTCCCGCCTGATGCATTCCC-
TGCTTAGAACTGACCAAGATCAGATGGAGGCTGCCATGAGCACAATGGAT-
GGTATCATTGACACAGTCCTGCAGTCGACCTCTAGAGCCTTGATT-
CATTGTTGAAGACTAATGGAAAAGTTGTTACCGTTGGTATAGCAGTGCAGC-

CACTCGATCTCCCAGTTTCCCTTGATAATAGGAAGGAAGATGGTAGCTG-
GTAGTGCATTGGAGGTATGAAAGAGACGCAAGAGATGATTGATTGCTG-
CTGAACATAACATAACAGCTGACATCGAGGTATCCGATTGATTACCT-
GAACACCGCAATGGAACCGCGTTGTCAAAAAAGATGTCAGGTTCGATTGT-
CATCGACGTTGAGAACACATTGTAAGTCCGCCTAAGTTTCATTCAATTCT-
GTTAATAAGACTATGCATTAATATATGACTGACTCTCCATAGGATGGAGT-
TATCAGTCTCAAATTCTAGACATATTTGTGATCAAATAATGGAATGGC-
TTTGTTCCTTCCACTAAGATTAGATTTCAGTTGTATTGTTAAAGA-
GATTGATGTTTATTAAATTGTAACAGTGTATCAGTCTAATCAT-
TAAAAAAAAAAAAAAA

INFORMATION FOR SEQ ID NO: 7B (SLF193)

SEQUENCE CHARACTERISTICS:

LENGTH: 326

TYPE: partial peptide

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 7B

ETGATDVRFKVLYCGVCHSDIHMAKNDWGTSTYPIVPGHELGVVTEVG-
CKVKKFKSWRQGRCWLGRLRPTCENCIHHLENYCPNLIQTYGSKYYDGTM-
TYGGYSNNMVTDEHFIVRIPDNLPLDGAAPLLCAGITTYSPWRYYGLDKPGM-
HLGVEWPRRFRSRPPLNLPGLWGSRLQSLVPPLIKEEGGSYGTSPALMHSLRT-

DQDQMEAAMSTMDGIIDTVPAVRPLEPLISLLKTNGKVVTVGIAVQPLDLPVF-
PLIIGRKMVAGSAIGGMKETQEMIDFAAEHNITADIEVIPIDYLNTAMERVVKK-
DVRFRFVIDVENTL

INFORMATION FOR SEQ ID NO: 8A (SLF122)

SEQUENCE CHARACTERISTICS:

LENGTH: 1064

TYPE: partial cDNA

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 8A

GCAAAGTGCAAAAATTAAAGTTGGAGACAAAGTTGGTGTGGTGCTTGG-
TAGGCTCATGCAAAACTTGCACAGCTGTGCTAACGATTGGAGAACTACT-
GCCCAAACAGATAACAGACTTACGGCGCCAAGTACCTTGACGGAACAAAC-
CACATACGGCGGTTACTCTGACATCATGGTGGCGGATGAGGCCTTGTA-
ATCCGTATTCCGGACAACCTGCCTCTTGAGGGTGCTGCTCCTCTCCTATGTG-
CCGGAAATCACAACTTACAGTCCCCTGAGGTATTCGGACTTGACAAACC-
CGGCATGCATGTCGGGTGGTGGCCTGGCGGTTAGGCCATGTCGCGGT-
GAAGTTGCCAAGGCTTGGGGTTAATGTCACAGTGATCAGTACCTCCGC-
TAATAAGAAAGATGAAGCTATTAAACACCTTGGTGCTGATTCTTCTTGGT-
CAGTCGTGACCAAGATCAGATGCAGGCTGCCATGGAACATTGGACGGTAT-
CATCGACACAGTTCCGCAGTCCACCCCCCTCCCACCTTGATTAGTTATT-
GAAGGCTAATGGAAAGCTTGTATGGTGGAGCACCAGAGAACGCCACTT-
GAGCTACCAGTTTTCTTAATAATGGGAAGGAAGACTTAGCCGGTAG-

TAATATCGGAGGTATCAAGGAGACACAAGAGATGATAGATTGGCAGC-
CAAACACAAACATAACGGCCGACATCGAGATTATCCCCATCGACTATTGAA-
CACTGCTATGGAGCGTCTGCTAAAGGGATGTTAGATACCGTTTGT-
CATCGACATCGGAAACACATTGAAGCCGCCATTAAATTGCATTCGAT-
CAGAAACTGAATCAAGCGAGGTGAGAGGCCTACGTAACAATGCAAACAT-
GTGCTAGCTTGTCTGGAGTAGTCTTAGCTTTCTGTATGTATTCCATC-
TGTGTTGTTCATGTCCCATCTTATTATGAGAAAAATGTGGGTACCGTGGGA-
TATTGAATAAATGAAGAGCTACTGGAACGATGGTTCA-
CAAAAAAAAAAAAAAAA

INFORMATION FOR SEQ ID NO: 8B (SLF122)

SEQUENCE CHARACTERISTICS:

LENGTH: 278

TYPE: partial peptide

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 8B

KVQKFKVGDKVGVGCLVGSCDKCDSCANDLENYCPKQIQTYGAKYLDGTT-
TYGGYSDIMVADEAFVIRIPDNLPLEGAAPLLCAGITTYSPLRYFGLDKPGM-
HVGVVGLGLGHAVKFAKALGVNVTVISTSANKDEAIKHLGADSLVSRD-
QDQMQAAMGTLDGIIDTVSAVHPLPPLISLLKANGKLVMVGAPEKPLELPVF-

SLIMGRKTLAGSNIGGIKETQEMIDLAAKHNTADIEIIPIDYLNTAMERLAKGD-
VRYRFVIDIGNTLKPAI

INFORMATION FOR SEQ ID NO: 9A (SLD194)

SEQUENCE CHARACTERISTICS:

LENGTH: 1228

TYPE: partial cDNA

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 9A

GCAAGAGATTCATCTGGTGTCCCTCTCCCTCAATTCTCCAGAAGG-
GAAACCGGAGAGAAAGACGTTATGTTCAAAGTGTGTACTGTGGAATTGCG-
CATTGGACCTTCACATGGTCAAGAACATGAATGGGGCTCTACCTATCCT-
TTGGTCCCAGGGCATGAGATTGTTGGTGAAGTTACGGAAGTAGGGAG-
CAAAGTACAAAAATTAAAGTTGGAGACAGAGTCGGTGTGGATGCGTTGT-
GGGATCTTGGCGATCTTGTGAAAATTGTACCGACCACCTTGAGAACTACTG-
CCCCAAACAGATACTCACTTACGGTGCCAAGTACTACGACGGAACCAC-
CTATGGCGGTTACTCTGACATTATGGTGGCCGACGAACACTTCATAGTACG-
CATCCCAGACAACCTGCCTCTTGATGGCGCTGCGCCGCTCCTATGTGCCGG-
GATTACAACCTACAGCCCCCTGAGATATTTCGGACTTGACAAGCCCAGG-
GCATGTAGGTGTGGTGGCCTAGGCCGTTAGGCCACGTCGCCGTGAAGT-
TTGCCAAGGCTATGGAGTGAAGGTTACAGTGATCAGTACGTCCCCTAA-

GAAAGAGGAGGAAGCTCTTAAACACCTAGGAGCTGACTCGTTTCGTTAG-
CCGTGACCAAGATCAAATGCAGGCTGCCATTGGTACCATGGATGGGAT-
CATTGACACAGTTCTGCACAACATCCTCTCCTGCCTTGATTGGTTGTT-
GAAGTCTCATGGAAAGCTTGTATGGTGGTGCACCAGAGAAGCCTCTT-
GAACCTCCAGTTTCCTTACTCATGGGAAGAAAGATGGGTAGCTGG-
TAACCGGCATTGGGGGTATGAAGGAGACACAAGAGATGATAGATTTGC-
TGCCAGGCACAACATAACAGCAGACATCGAAGTCATACAATCGACTACT-
TAAACACTGCTATGGAGCGTTAGTCAAAGCAGATGTCAGATAACGTTT-
GTCATCGACATTGGAAACACACTGAAGGCTAGCACTTAAATTCTGCAATC-
CAGACTGTATCAATGAAGAAACAAGAACAGAAACTGAGATTGATTGGTGT-
CATACTCCGCCTATGGTTTCCTTACAGCATTGTTGCTACATGA-
ATAACGATCACATGAACTGTGATGATTGATAATAAAAGAATAACATAAA-
CAAAAAAAACAAAAAAACAAAAAAACAAAAAA

INFORMATION FOR SEQ ID NO: 9B (SLD194)

SEQUENCE CHARACTERISTICS:

LENGTH: 284

TYPE: partial Peptide

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 9B

ARDSSGVLSPFNFSRRETGEKDVMFKVLYCGICHSDLHMVKNEWGFSTYPL-
VPGHEIVGEVTEVGSKVQKFKVGDRVGVCVGSCRSCENCTDHLENYCP-

KQILTYGAKYYDTTYGGSIDIMVADEHFIVRIPDNPLLDGAAPLLCAGIT-
TYSPLRYFGLDKPGMHGVVGLGLGHVAVKFAKAMGVKVTVISTSPK-
KEEEALKHLGADSFFVSRDQDQMQAIGTMDGIIDTVSAQHPLLPLIGLLKSH-
GKLVMVGAPEKPLELPVFPLLMGRKMGSW

INFORMATION FOR SEQ ID NO: 10A (SLF17)

SEQUENCE CHARACTERISTICS:

LENGTH: 852

TYPE: partial cDNA

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 10A

GTCCCCTGAGGTATTCGGACTTGACAAACCCGGCATGCATGTCGGGGTG-
GTTGGCCTTGGCGGTTAGGCCATGTCGCGGTGAAGTTGCCAAGGCTTG-
GGGGTTGAGGTCACAGTGATCAGTACCTCCGCTAATAAGAAAGATGAAGC-
TATTAAACACCTTGGTGTGATTCTTCTTGGTCAGTCGTGACCAAGATCA-
GATGCAGGCTGCCATGGAACATTGGACGGTATCATCGACACAGTTCTG-
CAGTCCACCCCTCCCACCTTGATTAGTTATTGAAGGCTAATGGAAAGC-
TTGTTATGGTTGGAGCACCAGAGAAGCCACTTGAGCTACCAGTTTTCTT-
TAATAATGGGAAGGAAGACTTGTGAGCTTGGCAGCTAACACAAACATAACGGCCGA-
GAGACACAAGAGATGATAGATTGGCAGCTAACACAAACATAACGGCCGA-
CATCGAGGTCACTCCCCATCGATTATTGAACACTGCAATGGAGCGTCTTGC-

TAAAGGGGATGTTAGATACCGGTTGTCATCGACATCGGAAACACATT-
GAAGCCGGCCACTTAAATTGCATTCGATCAGAAACTGAATCAAGCGAT-
GTCGAGAGGCCTACGTAACAATGTAAACATGTGCTAGCTTGTCTGTAG-
TAGTCTTAGCATTCTCTGATGTACTCCTCTGTTTGTTCATGTTCCATCT-
TATAATAAGATTCTTATTATGAAAAAAATATGGTACCGTGGATATTGA-
ATAAAATGAAGAACTACTGGAACAATGGTTCACAAATTATTGTGGTGC-
TAAAAA

INFORMATION FOR SEQ ID NO: 10B (SLF17)

SEQUENCE CHARACTERISTICS:

LENGTH: 188

TYPE: partial peptide

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DESCRIPTION FOR SEQ ID NO: 10B

PLRYFGLDKPGMHGVVGLGLGHAVKFAKALGVEVTVISTSANKDEAIK-
HLGADSLVSRDQDQMQAAMGTLDGIIDTVSAVHPLPPLISLLKANGKLVM-
VGAPEKPLELPVFSLIMGRKTLAGSNIGGIKETQEMIDLAAKHNTA-
DIEVIPIDYLNTAMERLAKGDVRYRFVIDIGNTLKPAT

INFORMATION FOR SEQ ID NO: 11A (SLF138)

SEQUENCE CHARACTERISTICS:

LENGTH: 664

TYPE: partial cDNA

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 11A

AGTTTGGTCTTGATGTGGGTGGATTAAGGGGAGGGATATTGGGACTTG-
GAGGTGTTGGACACATGGGGGTGAAGATAGCAAAGGCTATGGGACAC-
CATATCACCGTGATAAGCTCTTGATAAGAAGAAAAAGAGGCCTTGGAG-
CATATTGGTGCTGATGAGTACTTGGTGAGCTCTGATGCCACCCAAATGCAA-
GAGGCTATGGACTCACTGGATTACATTATTGACACCATTCCAGTGTCCAC-
CCTCTTGAGCCTTACCTCTCTTGTGAAGCTTGATGGGAAGTTGATCTT-
GATGGGTGTTATCAACACCCATTGCAATTGTCTCTCCATTGGTCATGCTT-
GGGGAGGAAGACGATCACCGGGAGCTTGTGGGGAGCATGAAGGAGATG-
GAGGAGATGCTCGAGTTCTGCAAAGAGAAAGAGCTGAAACGATGATT-
GAAGTGGTGAAGATGGACTACATCAACGAAGCTTCGAAAGGTTGGAGAA-
GAACGACGTTAGGTACAGGTTCGTTGTGGATTGTTGCCGGCAGCAATCTT-
GATCAATAAGAAAGAAAGAAGGCATCATCGAGTGTGTCCTATTAA-
TATCGAGTACTCTGTCTCATCTTATCTTAAACAATATAAAATAACAAA-
GAAAAAAAAAAAAAAAAAAAAAA

INFORMATION FOR SEQ ID NO:11B (SLF138)

SEQUENCE CHARACTERISTICS:

LENGTH: 181

TYPE: partial Peptide

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 11B

FGLDVGGLRGGLGLGGVGHMGVKA
KAMGHHITVISSSDKKKEALEHI-
GADEYLVSSDATQM**QEAMDSLDYI**IDTIPVFHPLEPYLSLLKLDGKLILM-
GVINTPLQFVSPLVMLGEEDDHRELC**GEHEGDGGDAR**VLQRERAETM**IEVVK**-
MDYINEAFERLEKNDVRYRFVVDCRQQS

INFORMATION FOR SEQ ID NO: 12A (SLG16)

SEQUENCE CHARACTERISTICS:

LENGTH: 694

TYPE: partial cDNA

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 12A

GTGCATTGCTATGCCTATGAAGGCAAGATGCAAGAACATCTGCAATTATGT-
GAAGACGAGTTAAAAAGATAATGAAGATAAATTCTATGTCTGCATGGTT-
TCTGGTAAATGCCGTTGGCAGAAGAATGCGAGATCATAAATCAGGAGGTT-
CATCATATTGTTGACCTCGATTGTTGGAGCTGAAAGAGGGCTTATACAG-
GAGCTGTTGCCTATGGTGCATGTTGGCAGCAGCACTGCAGCAGTTAGTAAG-
GTCGTCGGCATTGGAGATTGGAAAATACCAAGATCAGGGTTAATGCAATCG-
CACGTGGTTGCATTGGAAGATGAGTTCTAAGTCTGTGGGAATAGAGA-
GAGCAAAGAAGCTGGTGAATGATGCAGTTCCGCTGGAGAGATGGCTTGAT-
GTTAAAAATGATGTGGCTTCAAGTGTATATATTGGTCAGTGATGGTT-
CAAGGTACATGACGGGCACAACATATTGTTGATGGGCACAGTCTC-
TCGTGAGGCCTCGAATGCGTTCTTATATGTGATTCTGCTCCTATTATCC-
TCCTAGCCATTATTAGCTACTTAGGTTGTCATACTCATAGGTGAACCT-
CATTAGCTATTCTACATTGTTCTTATGAATAAAGAAGTCAAGATT-
CAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

INFORMATION FOR SEQ ID NO: 12B (SLG 16)

SEQUENCE CHARACTERISTICS:

LENGTH: 176

TYPE: partial Peptide

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 12B

VHCYAYEGKMQEHLQLCEDEFKKIMKINFMSAWFLVNAVGRMRDHKS GG-
SIILLTSIVGAERGLYTGA VAYGACSAALQQLVRSSALEIGKYQIRVNAIARGL-
HLEDEFPKSVGIERAKKL VNDAVPLERWLDVKNDVASSVIYL VSDGSRYMT-
GTTIFVDGAQSLVRPRMRSYM

INFORMATION FOR SEQ ID NO: 13A (SLG144)

SEQUENCE CHARACTERISTICS:

LENGTH: 1010

TYPE: partial cDNA

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DISCRIPTION FOR SEQ ID NO: 13A

GGAAACTACCATCAATTTGGGTCTAAGAAGATTGCAGTTGTTACTGGAGC-
CAACAAAGGGATTGGACTTGAGATTAGCAAGCAATTAGCTGCTAAAG-
GAGTTGGGTGGTATTAACAGCAAGAGATGTGAAGAGAGAGAACAGAACGCT-
GCTGAAAATCTTAAGGCTTCTGGGTCTCTGATGTGGTATTCATCAGCTA-
GATGTAACAGAGCCGACTACTATTGGTTCTTGGCAAACCTTCTGAAACG-
CAATTGGAAAGCTTGACATATTGGTTAACATGCAGGAGTCGTTGGATCT-
GTATACCTCACAGCCGACTATGATCCAGTGCAAACATACGAGACAGCGAG-
GGATTGTTGAAAACAAACTATTATGGGCTCAAGCAAGTCACAGAACGAC-
TTGTTCCGCTGCTCAAAATCTGAAGCTGCAAGGATAGTCAATGTCTCTC-
CGGATTAGGACAGCTAAGAAATATTGGAAATGAGAAGGCCAAGAAGGAGC-
TAGGAGATGCAGATAACCTCAACGAGGAGAAAGTGGACAAGCTAGTTGAG-
GAATTCTGGAGGATGTGAAACAGGGATTGATAGAATCCAAAGGCTGGCC-
TCTAAGTATATCTGCCTACATTGTCTCAAAAGCAGCTCTGAATGCTTATA-
CAAGACTCTGGCAAAGAAGTATCCCCATATTGCCATAACGCAGTTGGTC-
CAGGTTATACCAAAACAGACCTCAATAATAATTCCGGGATTCTCACAGTT-

GAAGAAGCTGCAGTAGGTCTGTGAGGCTGGCTTGATAGCCGAAACTA-
GAATTCCGGCCTCTTCTTCAACAGAAATGAAGAGTCGACCTTGATTAG-
GTCAACGTGATCCCTGATGAACGGACTATTTAGATTTCAGAATGTGC-
TTGATTTGTTGAAGTATTATGGGATTTGTATGTATACTTGATGTAT-
CATTGTATTAATAGAGCACATGTTGTGAT-
CAAAAAAAAAAAAAAAAAAAAAAA

INFORMATION FOR SEQ ID NO: 13B (SLG144)

SEQUENCE CHARACTERISTICS:

LENGTH: 284

TYPE: partial Peptide

STRANDNESS: Single

TOPOLOGY: Linear

OTHER INFORMATION: STRAWBERRY ALCOHOL DEHYDROGENASE

SEQUENCE DESCRIPTION FOR SEQ ID NO: 13B

ETTINFGSKKIAVVTGANKGIGLEISKQLAAKGVGVVLTARDVKRGTEAAENL-
KASGFSDVVFHQLDVTEPTTIGSLANFLETQFGKLDILVNNAGVVGSVYL-
TADYDPVQTYETARDCLKTNYYGLKQVTEALVPLLQKSEAARIJVNVSSGL-
GQLRNIGNEKAKKELGDADNLNEEKVDKLVEEFLEDVKQDSIESKGWPLSI-
SAYIVSKAALNAYTRLLAKKYPHIAINAVGPGYTKTDLNNNSGILTVEEAAGV-
PVRLALIAETRISGLFFNRNEESTFD

INFORMATION FOR SEQ ID NO: 14B (Motif 1)

SEQUENCE CHARACTERISTICS:

LENGTH: 12

TYPE: amino acid

STRANDNESS: Single

TOPOLOGY: Linear

HYPOTHETICAL SEQUENCE: NO

SEQUENCE DISCRIPTION FOR SEQ ID NO: 14B

Trp Thr Asn Phe Phe Asn Pro Leu Asp Phe Gly Trp
W T N F F N P L D F G W

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12.03.1999

CLAIMS

(58)

1. An isolated DNA sequence encoding
 - (a) a polypeptide having an amino acid sequence as shown in SEQ ID NO: 1B or SEQ ID NO: 2B, or
 - 5 (b) a polypeptide having at least 30% homology with the amino acid sequence SEQ ID NO: 1B or at least 40% homology with the amino acid sequence SEQ ID NO: 2B, or
 - (c) a fragment of polypeptide (a) or (b),
10 which polypeptide or fragment of polypeptide has alcohol acyl transferase activity and is involved in the biosynthetic pathway for aliphatic and /or aromatic ester production in fruit.
- 15 2. An isolated DNA sequence according to claim 1 encoding a polypeptide having at least 50% homology with any of the amino acid sequences SEQ ID NO: 1B or SEQ ID NO: 2B, or encoding a fragment thereof.
3. An isolated DNA sequence according to claim 1 or 2
20 encoding a polypeptide having at least 70% homology with any of the amino acid sequences SEQ ID NO: 1B or SEQ ID NO: 2B, or encoding a fragment thereof.
4. An isolated DNA sequence having a nucleic acid sequence
25 (a) as shown in SEQ ID NO: 1A or SEQ ID NO: 2A which encodes a polypeptide having alcohol acyl transferase activity and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, or
- 30 (b) complementary to SEQ ID NO: 1A or SEQ ID NO: 2A, or
- (c) which has at least 25% homology with any of the sequences of (a) or (b), or

(d) which is capable of hybridising under stringent conditions to any of the sequences (a) - (c) .

5. An isolated DNA sequence according to claim 4 having a nucleic acid sequence

5 (c) which has at least 40% homology with any of the sequences of (a) or (b) , or

(d) which is capable of hybridising under stringent conditions to said sequence (c) .

6. An isolated DNA sequence according to claim 4 having a nucleic acid sequence

10 (c) which has at least 60% homology with any of the sequences of (a) or (b) , or

(d) which is capable of hybridising under stringent conditions to said sequence (c) .

15 7. A purified and isolated polypeptide,

(a) having an amino acid sequence as shown in SEQ ID NO: 1B or SEQ ID NO: 2B, or

(b) having at least 30% homology with the amino acid sequence SEQ ID NO: 1B or at least 40% homology with the amino acid sequence SEQ ID NO: 2B, or

20 (c) being a fragment of polypeptide (a) or (b) , which polypeptide or fragment of polypeptide has alcohol acyl transferase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

25

8. A purified and isolated polypeptide according to claim 7 having at least 50% homology with any of the amino acid sequences SEQ ID NO: 1B or SEQ ID NO: 2B, or a fragment thereof.

30

9. A purified and isolated polypeptide according to claims 7 or 8 having at least 70% homology with any

of the amino acid sequences SEQ ID NO: 1B or SEQ ID NO: 2B, or a fragment thereof.

10. A recombinant expression vector comprising a coding sequence which is operably linked to a promoter

5 sequence capable of directing expression of said coding sequence in a host cell for said vector, and a transcription termination sequence, in which the coding sequence is a DNA sequence as defined in any of claims 1 to 6.

10 11. A replicative cloning vector comprising an isolated DNA sequence as defined in any of claims 1 to 6 and a replicon operative in a host cell for said vector.

12. A method for regulating aliphatic and/or aromatic ester formation in fruit, comprising inserting into 15 the genome of a fruit-producing plant one or more copies of one or more DNA sequences as defined in any of claims 1 to 6.

13. A method as claimed in claim 12 in which the plant 20 is strawberry, citrus (lemon), banana, apple, pear, melon, tomato, sweet pepper, peach or mango.

14. A plant and propagating material thereof which contains within its genome a vector as defined in claim 10 or 11.

25 15. A genetically modified strawberry or lemon plant and propagating material derived therefrom which has a genome comprising an expression vector for over-expression or down-regulation of an endogenous strawberry or lemon plant gene counterpart of any 30 of the DNA sequences as defined in claims 1 to 6.

16. A method for producing aromatic and/or aliphatic esters in microorganisms, plant cells or plants comprising inserting into the genome of the microorganism or plant one or more copies of DNA sequences as defined in any of claims 1 to 6, and feeding an alcohol and an acyl-CoA to the microorganism or plant (cell).

5

17. An isolated DNA sequence encoding

10 (a) a polypeptide having an amino acid sequence as shown in SEQ ID NO: 3B, or

(b) a polypeptide having at least 80% homology with the amino acid sequence SEQ ID NO: 3B, or

15 (c) a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has aminotransferase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

18. An isolated DNA sequence having a nucleic acid sequence

20 (a) as shown in SEQ ID NO: 3A which encodes a polypeptide having aminotransferase activity and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, or

25 (b) complementary to SEQ ID NO: 3A, or

(c) which has at least 70 % homology with any of the sequences of (a) or (b), or

(d) which is capable of hybridising under stringent conditions to any of the sequences (a) - (c).

30 19. A purified and isolated polypeptide,

(a) having an amino acid sequence as shown in SEQ ID NO: 3B, or

(b) having at least 80% homology with the amino acid sequence SEQ ID NO: 3B, or

5 (c) being a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has aminotransferase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

20. An isolated DNA sequence encoding
(a) a polypeptide having an amino acid sequence as shown in SEQ ID NO: 4B, or
(b) a polypeptide having at least 90% homology with the amino acid sequence SEQ ID NO: 4B, or
10 (c) a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has thiolase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.
15

21. An isolated DNA sequence having a nucleic acid sequence
(a) as shown in SEQ ID NO: 4A which encodes a polypeptide having thiolase activity and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, or
20 (b) complementary to SEQ ID NO: 4A, or
(c) which has at least 75 % homology with any of the sequences of (a) or (b), or
25 (d) which is capable of hybridising under stringent conditions to any of the sequences (a) - (c).
30

22. A purified and isolated polypeptide,
(a) having an amino acid sequence as shown in SEQ
30 ID NO: 4B, or
(b) having at least 90% homology with the amino acid sequence SEQ ID NO: 4B, or
(c) being a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has thiolase activity and is involved in the
35

biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

23. An isolated DNA sequence encoding
5 (a) a polypeptide having an amino acid sequence as shown in SEQ ID NO: 5B, or
(b) a polypeptide having at least 90% homology with the amino acid sequence SEQ ID NO: 5B, or
(c) a fragment of polypeptide (a) or (b),
10 which polypeptide or fragment of polypeptide has pyruvate decarboxylase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

24. An isolated DNA sequence having a nucleic acid sequence
15 (a) as shown in SEQ ID NO: 5A which encodes a polypeptide having pyruvate decarboxylase activity and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, or
20 (b) complementary to SEQ ID NO: 5A, or
(c) which has at least 75 % homology with any of the sequences of (a) or (b), or
(d) which is capable of hybridising under stringent
25 conditions to any of the sequences (a) - (c).

25. A purified and isolated polypeptide,
30 (a) having an amino acid sequence as shown in SEQ ID NO: 5B, or
(b) having at least 90% homology with the amino acid sequence SEQ ID NO: 5B, or
(c) being a fragment of polypeptide (a) or (b),
35 which polypeptide or fragment of polypeptide has pyruvate decarboxylase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

26. An isolated DNA sequence encoding
(a) a polypeptide having an amino acid sequence as
shown in SEQ ID NO: 6B, or
(b) a polypeptide having at least 75% homology with
the amino acid sequence SEQ ID NO: 6B, or
(c) a fragment of polypeptide (a) or (b),
which polypeptide or fragment of polypeptide
has alcohol dehydrogenase activity and is
involved in the biosynthetic pathway for ali-
phatic and/or aromatic ester production in
fruit.

5

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27. An isolated DNA sequence having a nucleic acid
sequence
(a) as shown in SEQ ID NO: 6A which encodes a
polypeptide having alcohol dehydrogenase acti-
vity and being involved in the biosynthetic
pathway for aliphatic and/or aromatic ester
production in fruit, or
15

20

(b) complementary to SEQ ID NO: 6A, or
(c) which has at least 65 % homology with any of
the sequences of (a) or (b), or
(d) which is capable of hybridising under stringent
conditions to any of the sequences (a) - (c).

28. A purified and isolated polypeptide,
(a) having an amino acid sequence as shown in SEQ
ID NO: 6B, or
25

(b) having at least 75% homology with the amino
acid sequence SEQ ID NO: 6B, or
(c) being a fragment of polypeptide (a) or (b),
30

which polypeptide or fragment of polypeptide
has alcohol dehydrogenase activity and is
involved in the biosynthetic pathway for ali-
phatic and/or aromatic ester production in
fruit.

35 29. An isolated DNA sequence encoding

(a) a polypeptide having an amino acid sequence selected from the group consisting of sequences SEQ ID NO: 7B, 8B, 9B and 10B, or

(b) a polypeptide having at least

5 i) 55% homology with the amino acid sequence of a 326 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 7B, or

10 ii) 75% homology with the amino acid sequence of a 278 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 8B, or

15 iii) 65% homology with the amino acid sequence of a 284 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 9B, or

20 iv) 80% homology with the amino acid sequence of a 188 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 10B, or

(c) a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has alcohol dehydrogenase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

30. An isolated DNA sequence having a nucleic acid sequence

(a) selected from the group consisting of sequences SEQ ID NO: 7A, 8A, 9A and 10A, which encodes a polypeptide having alcohol dehydrogenase activity and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, or

30 (b) complementary to SEQ ID NO: 7A, 8A, 9A or 10A, or

- (c) which has at least 55% homology with any of the sequences of (a) or (b), or
- (d) which is capable of hybridising under stringent conditions to any of the sequences (a) - (c).

5 31. An isolated DNA sequence according to claim 30 having a nucleic acid sequence

- (c) which has at least 65% homology with any of the sequences of (a) or (b), or
- (d) which is capable of hybridising under stringent conditions to said sequence (c).

10

32. A purified and isolated polypeptide,

- (a) having an amino acid sequence selected from the group consisting of sequences SEQ ID NO: 7B, 8B, 9B and 10B, or

15

- (b) having at least

- i) 55% homology with the amino acid sequence of a 326 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 7B

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- ii) 75% homology with the amino acid sequence of a 278 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 8B, or

25

- iii) 65% homology with the amino acid sequence of a 284 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 9B, or

30

- iv) 80% homology with the amino acid sequence of a 188 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 10B, or

35

- (c) being a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has alcohol dehydrogenase activity and is involved in the biosynthetic pathway for ali-

phatic and/or aromatic ester production in fruit.

33. An isolated DNA sequence encoding

5 (a) a polypeptide having an amino acid sequence as shown in SEQ ID NO: 11B, or

10 (b) a polypeptide having at least 75% homology with the amino acid sequence of a 181 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 11B, or

15 (c) a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has alcohol dehydrogenase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

34. An isolated DNA sequence having a nucleic acid sequence

20 (a) as shown in SEQ ID NO: 11A which encodes a polypeptide having alcohol dehydrogenase activity and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, or

25 (b) complementary to SEQ ID NO: 11A, or

(c) which has at least 48 % homology with any of the sequences of (a) or (b), or

(d) which is capable of hybridising under stringent conditions to any of the sequences (a) - (c).

35. A purified and isolated polypeptide,

30 (a) having an amino acid sequence as shown in SEQ ID NO: 11B, or

(b) having at least 75% homology with the amino acid sequence of a 181 aa fragment from the 3' end of SEQ ID NO: 11B, or

35 (c) being a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide

has alcohol dehydrogenase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

5 36. An isolated DNA sequence encoding

- (a) a polypeptide having an amino acid sequence selected from the group consisting of sequences SEQ ID NO: 12B and 13B, or
- (b) a polypeptide having at least
 - i) 55% homology with the amino acid sequence of a 176 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 12B, or
 - ii) 35% homology with the amino acid sequence of a 284 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 13B, or
- (c) a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has alcohol dehydrogenase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

37. An isolated DNA sequence having a nucleic acid sequence

- (a) selected from the group consisting of sequences SEQ ID NO: 12A and 13A, which encodes a polypeptide having alcohol dehydrogenase activity and being involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit, or
- (b) complementary to SEQ ID NO: 12A or 13A, or
- (c) which has at least 20% homology with any of the sequences of (a) or (b), or
- (d) which is capable of hybridising under stringent conditions to any of the sequences (a) - (c).

38. An isolated DNA sequence according to claim 37 having a nucleic acid sequence

5 (c) which has at least 30% homology with any of the sequences of (a) or (b), or

(d) which is capable of hybridising under stringent conditions to said sequence (c).

39. A purified and isolated polypeptide,

10 (a) having an amino acid sequence selected from the group consisting of sequences SEQ ID NO: 12B and 13B, or

15 (b) having at least

i) 55% homology with the amino acid sequence of a 176 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 12B, or

ii) 35% homology with the amino acid sequence of a 284 aa fragment from the C terminal end of the coding sequence of SEQ ID NO: 13B, or

20 (c) being a fragment of polypeptide (a) or (b), which polypeptide or fragment of polypeptide has alcohol dehydrogenase activity and is involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit.

25

40. A method for regulating aliphatic and/or aromatic ester formation in fruit comprising inserting into the genome of a fruit-producing plant one or more copies of one or more DNA sequences as defined in any of claims 17, 18, 20, 21, 23, 24, 26, 27, 29, 30, 31, 33, 34, 36, 37 or 38.

35

41. A genetically modified strawberry or lemon plant and propagating material derived therefrom which has a genome comprising an expression vector for overexpression or downregulation of an endogenous

strawberry or lemon plant gene counterpart of any of the DNA sequences as defined in claims 17, 18, 20, 21, 23, 24, 26, 27, 29, 30, 31, 33, 34, 36, 37 or 38.

5 42. A method for producing aromatic and/or aliphatic esters in microorganisms, plant cells or plants comprising inserting into the genome of the microorganism or plant one or more copies of DNA sequences as defined in any of claims 1 to 6, and one or more copies of DNA sequences as defined in any of claims 26, 27, 29, 30, 31, 33, 34, 36, 37 or 38, and feeding aldehydes and acyl-CoA to the microorganism or plant (cell).

10

43. A method for producing aromatic and/or aliphatic esters in microorganisms, plant cells or plants comprising inserting into the genome of the microorganism or plant one or more copies of DNA sequences as defined in any of claims 1 to 6, and one or more copies of DNA sequences as defined in any of claims 26, 27, 29, 30, 31, 33, 34, 36, 37 or 38, and one or more copies of DNA sequences as defined in any of claims 23 or 24, and feeding alpha-keto acids and acyl-CoA to the microorganism or plant (cell).

15

20

25 44. A method for producing aromatic and/or aliphatic esters in microorganisms, plant cells or plants comprising inserting into the genome of the microorganism or plant one or more copies of DNA sequences as defined in any of claims 1 to 6, and one or more copies of DNA sequences as defined in any of claims 26, 27, 29, 30, 31, 33, 34, 36, 37 or 38, and one or more copies of DNA sequences as defined in any of claims 23 or 24, and one or more copies of DNA sequences as defined in any of claims

30

17 or 18, and feeding amino acids and acyl-CoA to the microorganism or plant (cell).

45. A method for producing aromatic and/or aliphatic esters in microorganisms, plant cells or plants comprising inserting into the genome of the microorganism or plant one or more copies of DNA sequences as defined in any of claims 1 to 6, and one or more copies of DNA sequences as defined in any of claims 26, 27, 29, 30, 31, 33, 34, 36, 37 or 38, and one or more copies of DNA sequences as defined in any of claims 23 or 24, and one or more copies of DNA sequences as defined in any of claims 17 or 18, and one or more copies of DNA sequences as defined in any of claims 20 en 21, and feeding amino acids and fatty acids to the microorganism or plant (cell).

46. An isolated DNA sequence according to any of claims 1 to 6 obtainable by cloning using as a primer a nucleotide sequence encoding the amino acid sequence as set forth in SEQ ID NO: 14B.

47. An antibody capable of specifically binding one or more polypeptides as claimed in any of claims 7-9, 19, 22, 25, 28, 32, 35 and 39.

48. A diagnostic kit for screening fruit with specific reference to volatile aliphatic and/or aromatic ester compounds comprising

25 a) one or more polypeptides as claimed in any of claims 7-9, 19, 22, 25, 28, 32, 35 and 39, or

b) one or more DNA sequences as claimed in any of claims 1-6, 17, 18, 20, 21, 23, 24, 26, 27, 29, 30, 31, 33, 34, 36, 37 and 38, or

30 c) one or more antibodies as defined in claim 47.

49. A method for screening fruit

- a) for fruit quality trait attributes, such as flavour, fragrance, aroma, scent, texture, shape, or
- b) to distinguish between cultivars/varieties of fruits based on their volatile ester profiles, or
- c) to monitor harvest time of fruit(s) for quality trait attributes, or
- d) to monitor post-harvest fruit(s) for quality trait attributes, or
- e) to monitor shelf-life of fruits, or
- f) to monitor timing for application of pesticides based on volatile ester profiles, or
- g) to monitor fruit resistance capacity based on their volatile ester profiles, which comprises using the diagnostic kit as claimed in claim 48.

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ABSTRACT

(58)

DNA sequences encoding enzymes involved in the biosynthetic pathway for aliphatic and/or aromatic ester production in fruit are disclosed. The enzymes have alcohol acyl transferase, alcohol dehydrogenase, pyruvate decarboxylase, thiolase and aminotransferase activities, respectively. The polypeptides having said activities are disclosed. Expression vectors comprising the DNA sequences may be used for regulating ester formation in fruit. Genetically modified plants and microorganisms may be used for producing esters.

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Fatty acids,
Amino acids

*amino-transferase,
thiolase*

thiolase

Short chain fatty acids,
 α keto acids

pyruvate decarboxylase

+ Coa

Aldehydes

alcohol dehydrogenase

Alcohols

Acyl - coA's

alcohol acyl transferase

Esters

figure 1

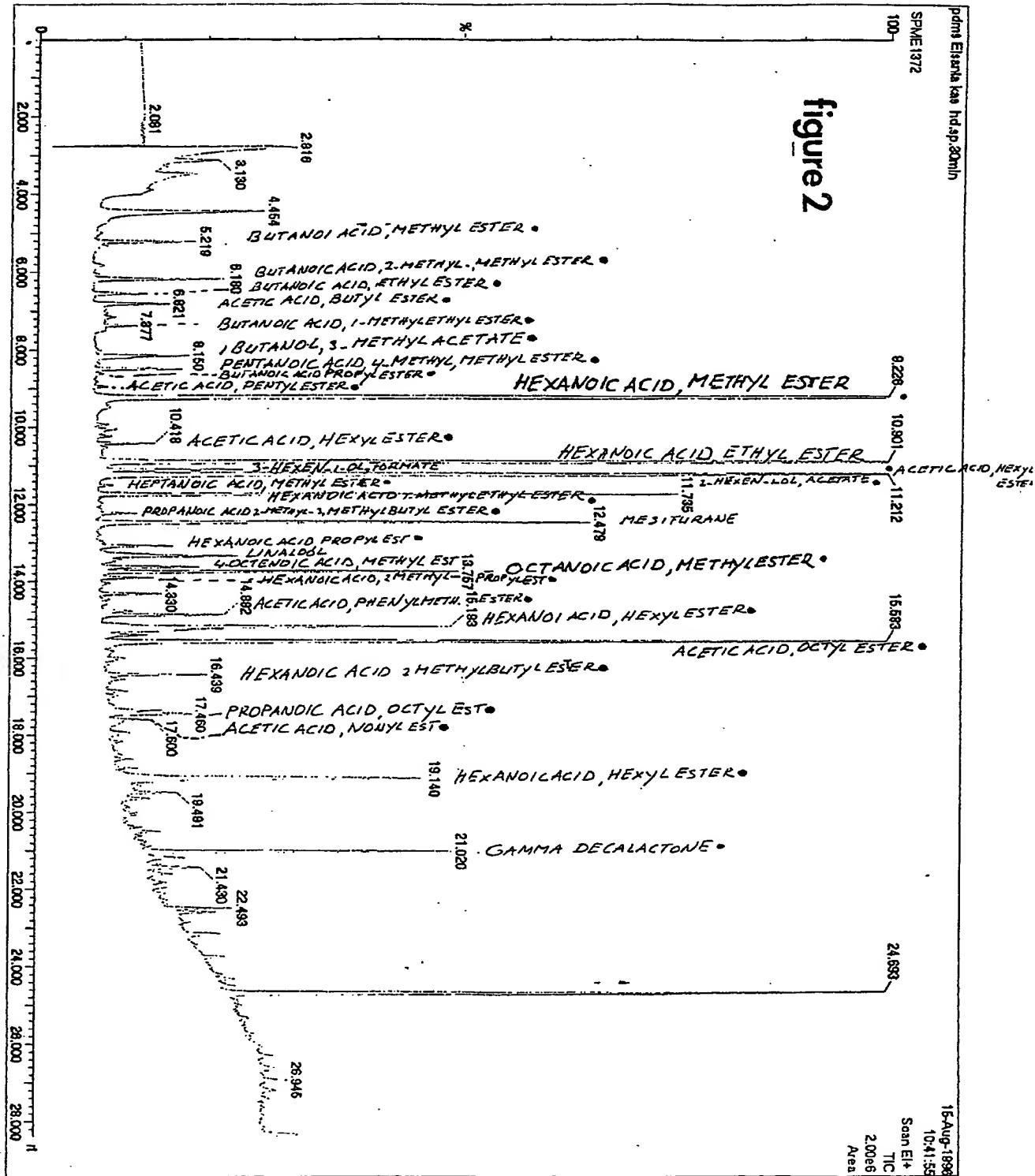


figure 2

**Strawberry alcohol acyl transferase expression
in the different strawberry plant tissues
(Northern blot analysis)**

1 2 3 4 5 6 7 8 9 10

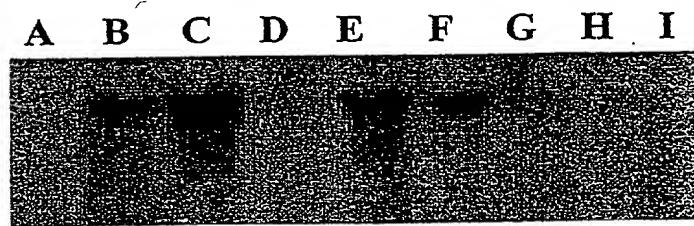


1 = root
2 = petiole
3 = leaf
4 = flower
5 = green fruit

6 = white fruit
7 = turning fruit
8 = red fruit
9 = seeds
10 = overripe fruit

figure 3

Citrus lemon acyl transferase expression in the different citrus plant tissues (Northern blot analysis)



A = root

B = leaf

C = flower bud

D = albedo

E = green fruit peel (1.2 x 2 cm)

F = green fruit peel (2 x 3 cm)

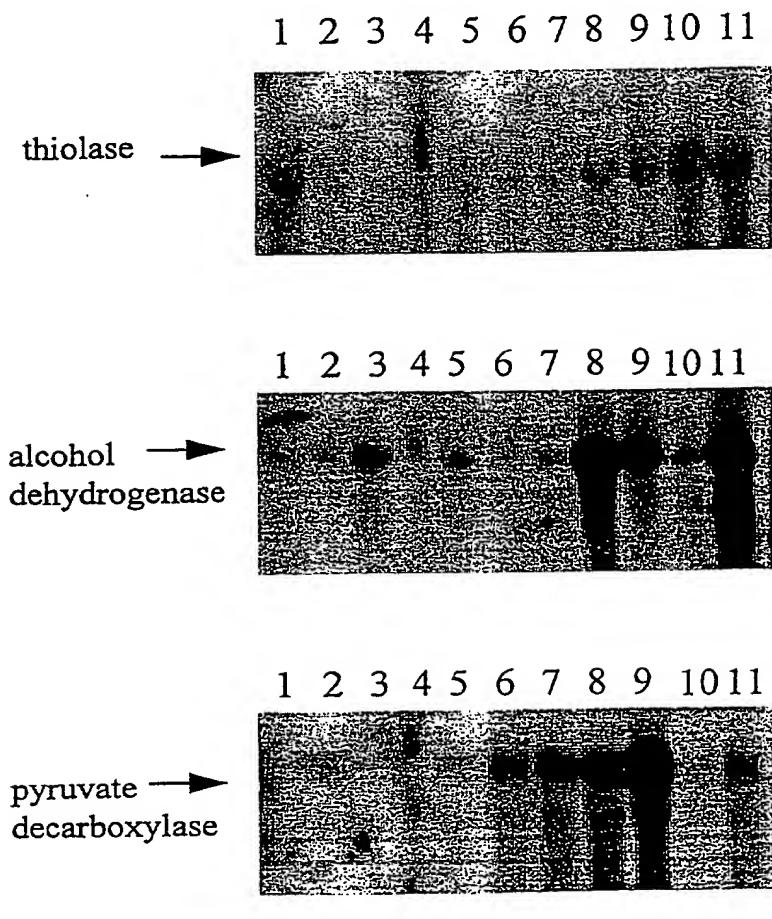
G = green fruit peel (3.5 x 6 cm)

H = green fruit peel (6 x 8 cm), ripe

I = yellow fruit peel (2 weeks detached)

figure 4

Expression of thiolase, alcohol dehydrogenase and pyruvate decarboxylase in different strawberry tissues



1 = root
2 = petiole
3 = leaf
4 = flower
5 = green fruit
6 = white fruit

7 = turning fruit
8 = red fruit
9 = red fruit without the seeds (achenes)
10 = seeds
11 = over ripe

figure 5

Southern blot analysis of strawberry alcohol acyl transferase

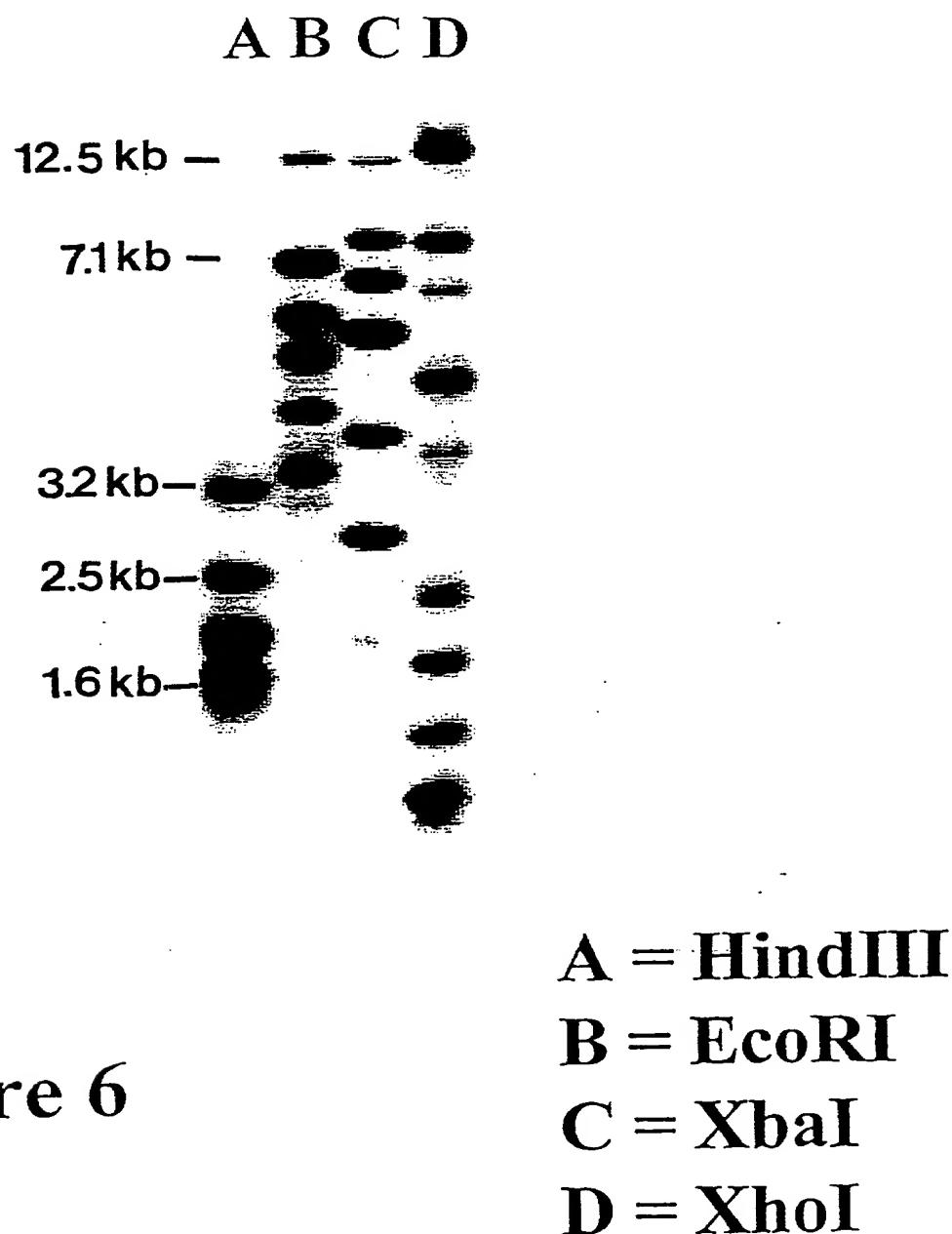


figure 6

The pRSET B expression vector used for the cloning of strawberry alcohol acyl transferase (E27)

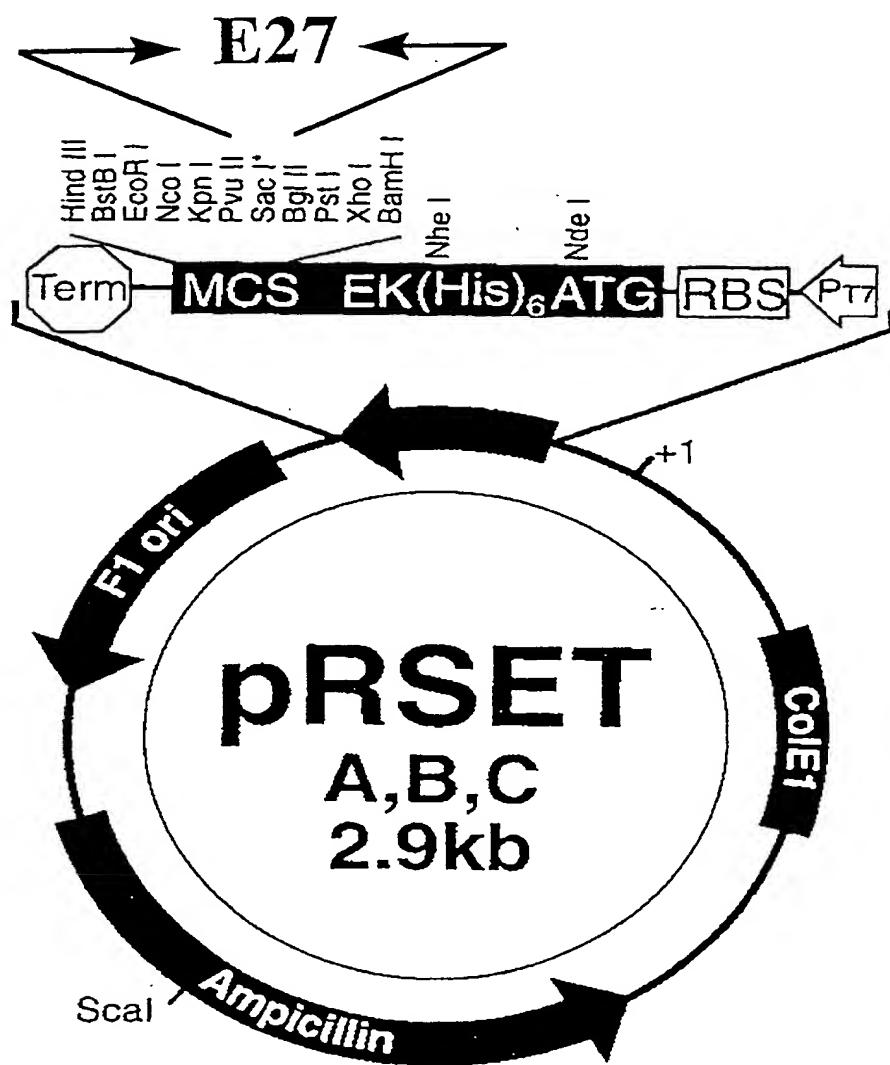
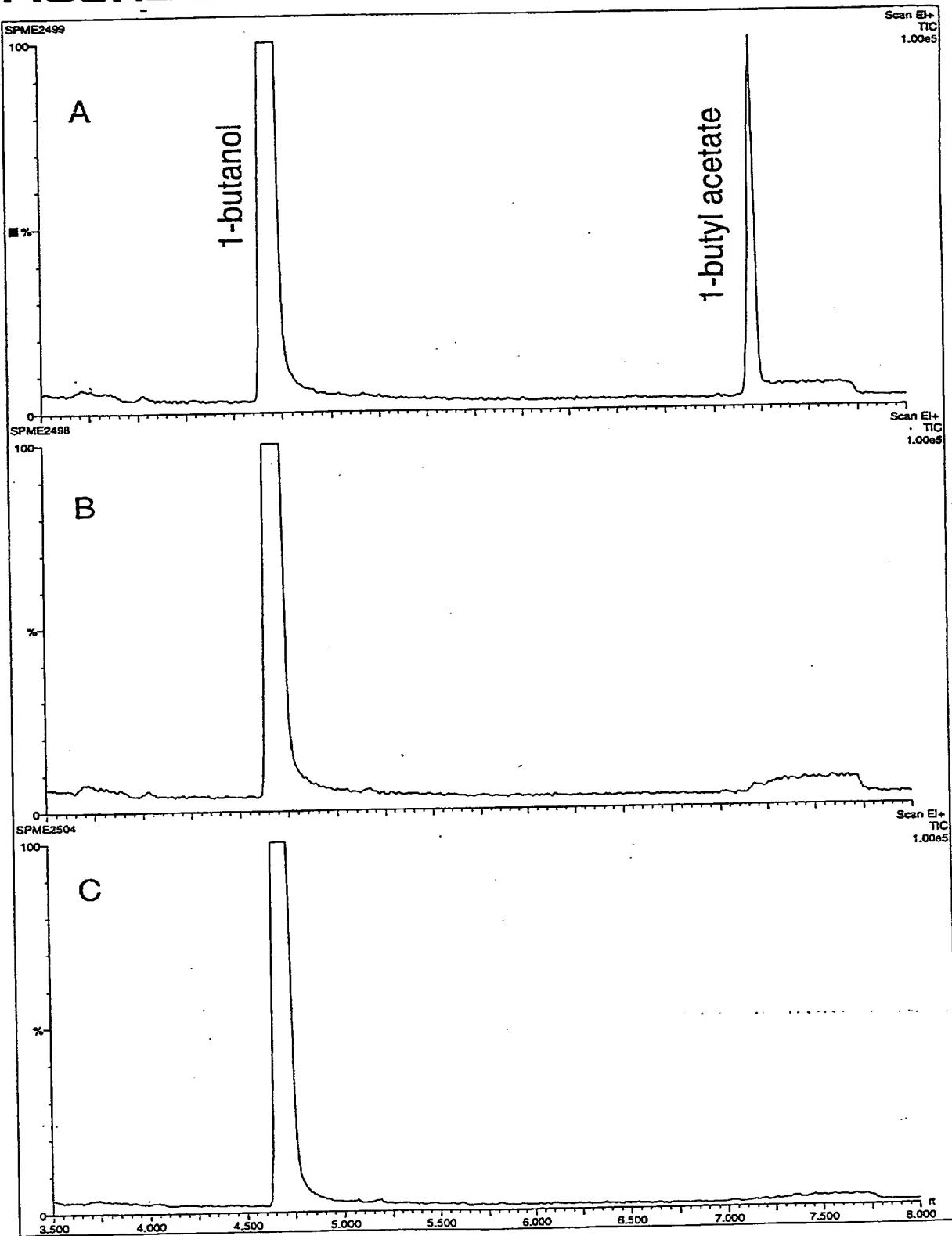
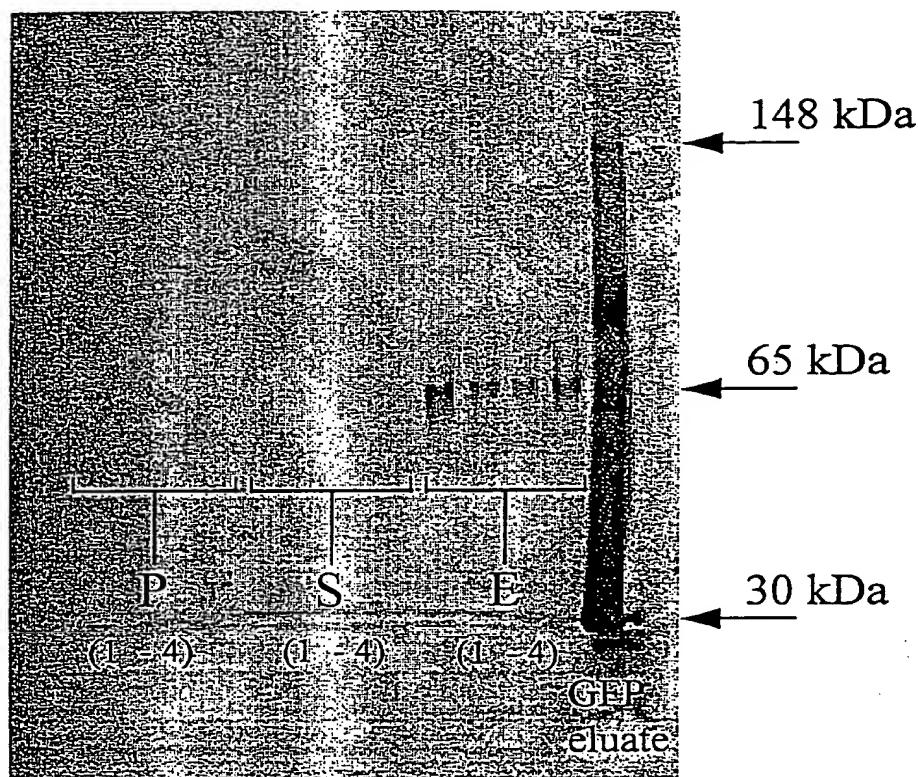


figure 7

FIGURE 8



**Western blot analysis with the commercial antibody
recognizing an epitope peptide fused to the N - terminus
of the recombinant strawberry alcohol acyl
transferase (E27).**



P = pellet from E27 cultures

S = supernatant from E27 cultures

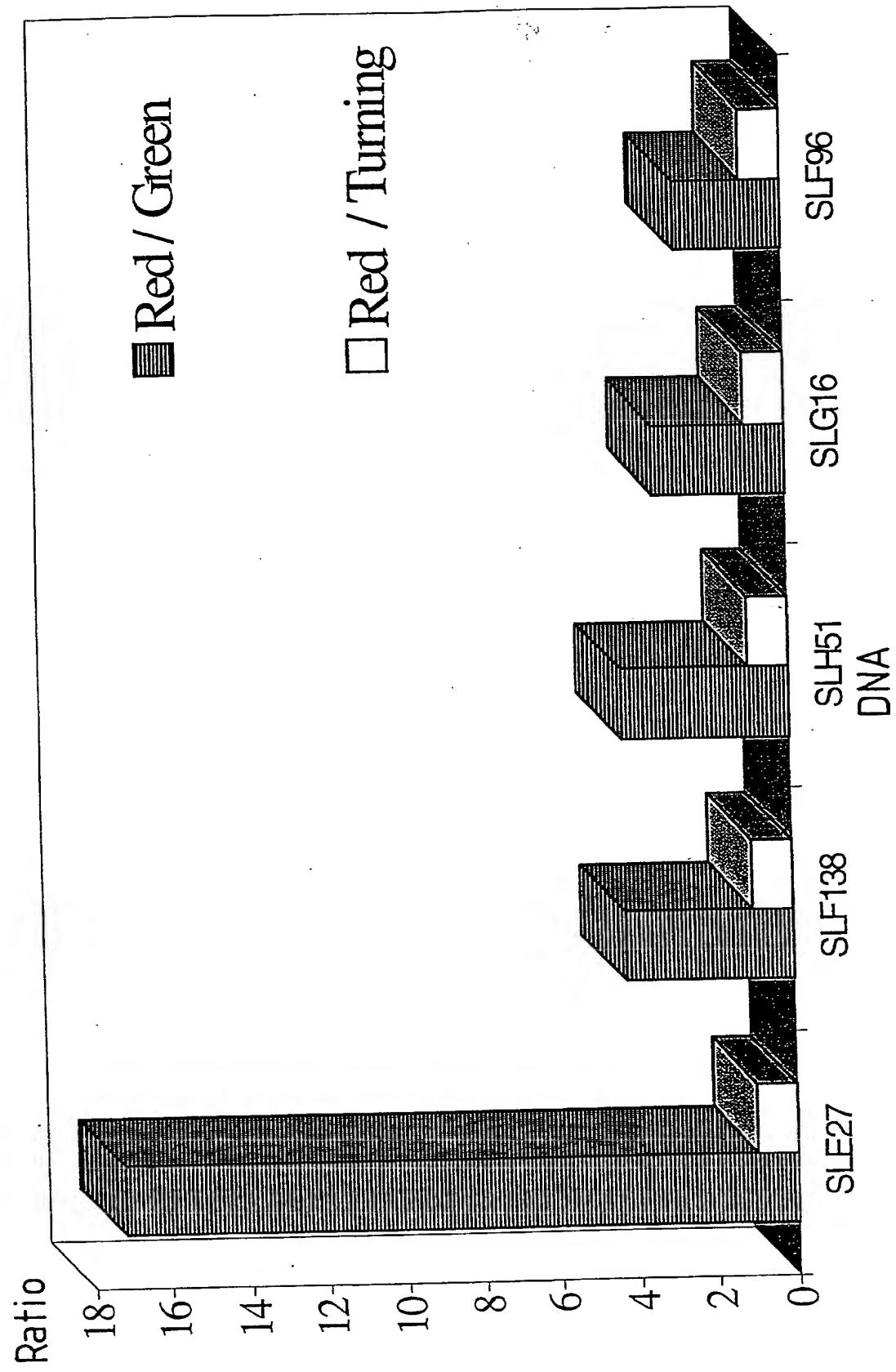
E = eluate from the Ni - NTA column from E27 cultures

GFP = recombinant Green Fluorescent Protein purified in
the same way as E27.

1 - 4 are four different growth and induction treatments

figure 9

figure 10



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